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EDITED BY

SIMON FLEXNER, M.D. PEYTON ROUS, M.D.

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EXPERIMENTAL BONE MARROW REACTIONS.

II. Normoblastosis Produced by India Ink. The Influence of Pregnancy on This Phenomenon.

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(Received for publication, October 21, 1926.)

In a previous investigation (1) it was found that a comparatively aplastic, fatal anemia was produced in rabbits by repeated injections of collargol. This was interpreted as the result of injury to those endothelial cells which are considered as the precursor cells of the erythrocytes and clasmatocytes.

The marked and rapid effect obtained with colloidal silver suggested that this substance exerted not only a mechanical but also a toxic action upon the widespread system of endothelial cells. It seemed desirable, therefore, to analyze the reaction of the blood and blood-forming organs to a comparatively inert substance in colloidal suspension. Carbon particles served the purpose, especially as it has been noted by various observers (2, 3) that carbon exhibits a special affinity for the endothelial cells.

Material and Methods.

As experimental animals twenty-one rabbits were used.

The investigation was begun with an India ink manufactured by F. Weber Co., Philadelphia. This product was found to exert a marked stimulation on the blood-forming organs. Therefore, in the attempt to isolate the factor in the ink possessing this stimulating effect on the bone marrow, experiments were made later with the filtrate of this ink, obtained by passing it through a Berkefeld filter W, thus removing practically all the carbon particles, as well as dialysate of the ink, obtained by suspending 100 cc. of the ink in a parchment bag in 50 cc. of distilled water, and allowing it to stand for 4 days. Other inks,—Higgins' Waterproof and General Drawing Inks, as well as Chin Chin and Pelican Waterproof Drawing Inks, manufactured by Günther Wagner, Hanover, were tried, in order to determine whether or not the effect produced was a property common to col-

loidal suspensions of carbon particles, obtained in the market in the form of drawing inks.

As the present investigation was intended as a control of a previous study with colloidal silver (1) the same experimental procedure has been employed, unless otherwise stated, *i.e.*, the substance used was diluted with equal quantities of distilled water, and injections were given intravenously every 2nd day as a rule, the dose varying from 2 to 10 cc. The changes in the blood were followed by examinations at frequent intervals. Zenker-fixed tissues, stained with eosin and methylene blue, were used for microscopical examinations. Counts were also made on supravital bone marrow preparations, as well as on fixed smears of the bone marrow.

The nomenclature of the blood cells used in this paper is the one employed by Sabin (4). In fixed smears the difficulty encountered in classifying the earliest blood cells designated by many as hemoblasts, is fully appreciated, but it is felt it can be done with reasonable certainty. In the tables, the very early erythroblasts, with characteristic nuclei but no demonstrable hemoglobin have been classified separately, to show to what extent these forms appeared in the peripheral blood. No cells that could be classified as definite megaloblasts were found in the peripheral circulation although present in all the hyperplastic marrows.

EXPERIMENTAL OBSERVATIONS.

Injections of Weber's Drawing Ink.

Most of the observations here reported are based on the intravenous injections of F. Weber Co.'s Waterproof Drawing Ink and its filtrate. The results obtained with this ink were remarkably constant, if one allows for small variations due to individual differences in the animals studied.

With large doses (6 to 10 cc. of a 50 per cent suspension) the peripheral blood showed the following changes: first a gradual decline often preceded by a slight rise of erythrocytes and hemoglobin. If the same dose was used at each injection, this decline was usually followed by a rise to practically the initial level. With gradually increasing doses it was possible to produce a moderate anemia in three instances, and in one (No. H-25), a severe anemia which ended fatally, with terminal counts of 1,420,000 erythrocytes, 18 per cent hemoglobin, and 18,000 nucleated red cells per c.mm. This animal was given 54 doses, gradually increased from 4 to 10 cc., and administered with increasing frequency, over a period of 114 days.

When smaller dosage was employed such as 4 cc. of a 50 per cent

solution, the hemoglobin and erythrocytes increased above the initial level. This increase was sustained for a considerable but variable time, then, with continued injections, the red blood cells and hemoglobin fell to, or below, the initial level. In one animal (No. H-60) receiving 4 cc. doses, the erythrocytes and hemoglobin gradually

TABLE I.

Rabbit H-24. Intravenous Injections of Weber's Drawing Ink Diluted with an Equal Quantity of Distilled Water. Large Dosage.

Date	Hemoglobin	Red blood cells	White blood cells	Normoblasts	Erythroblasts		Remarks
					With hemoglobin	No hemoglobin	
		per c.mm.					
1924	*per cent*						
Dec. 10	57	5,780,000	12,000	0	0	0	Injections begun
" 15	53	5,760,000	24,000	6,330	0	0	Dose 6 cc. three times a wk.,
" 17	48	4,730,000	9,400	16,650	273	0	except where otherwise
" 22	52	4,250,000	11,000	4,990	0	0	noted
" 27	47	3,610,000	13,400	20,880	286	0	
1925							
Jan. 1	46	4,230,000	18,000	12,200	200	0	
" 6	50	4,490,000	7,200	13,830	87	0	Dose 8 cc. injections given
" 10	55	5,310,000	15,900	38,790	954	0	every day from Jan. 5 to
" 15	49	3,890,000	14,000	54,660	8,410	0	15
" 20	48	4,870,000	5,500	37,760	3,360	60	
" 27	48	4,600,000	12,300	31,720	3,250	0	
Feb. 2	45	4,260,000	16,600	39,360	3,600	0	
" 10	44	4,270,000	13,100	11,310	130	0	
" 17	52	4,380,000	16,000	19,140	1,320	0	
" 25	52	4,390,000	23,000	35,220	3,690	246	
Mar. 2	54	4,620,000	12,500	3,430	0	0	Dose omitted Feb. 28
" 5	57	5,180,000	15,700	22,360	1,204	0	Killed

declined to about 50 per cent of normal, then a gradual increase was apparent especially in the hemoglobin.

The most striking and constant feature in all the animals studied, with large or small dosage, was the appearance, after one or two injections, of nucleated red blood cells in the peripheral blood. The number of nucleated cells increased as injections were continued, and in several instances reached high values (60,000 to 70,000 per c.mm.).

In the beginning of the course the nucleated red cells were primarily normoblasts; later however, erythroblasts of increasingly early stages appeared in the peripheral blood. This outpouring of nucleated red blood cells continued uninterrupted during the entire time that the animals were under observation, in two instances for 4 months. The

TABLE II.

Rabbit H-58. Intravenous Injections of Weber's Drawing Ink Diluted with an Equal Quantity of Distilled Water. Small Dosage. Illustrating Effect of Pregnancy.

Date	Hemoglobin	Red blood cells	White blood cells	Normoblasts	Erythroblasts		Remarks
					With hemoglobin	No hemoglobin	
		per c.mm.					
1925	*per cent*						
Sept. 29	78	6,770,000	13,900	0	0	0	Injection begun
Oct. 2	83	6,970,000	9,130	4,473	91	0	Dose 4 cc. three times a wk.
" 6	104	7,470,000	9,000	630	0	0	
" 10	92	6,980,000	5,900	12,962	236	0	
" 15	101	7,100,000	11,080	14,520	1,100	0	
" 20	80	6,890,000	5,600	12,840	560	0	
" 24	93	6,730,000	12,500	27,375	750	0	
" 29	87	7,370,000	17,600	24,288	1,232	0	
Nov. 3	92	7,040,000	10,240	8,058	102	0	One injection omitted Nov. 6
" 9	83	6,220,000	13,000	390	0	0	
" 17	71	5,130,000	8,300	0	0	0	
" 19	74	5,240,000	10,990	110	0	0	Litter of four young
" 24	70	5,280,000	12,440	10,788	3,968	248	Nov. 19. Blood count
Dec. 3	64	5,370,000	11,200	27,360	5,472	342	done after litter cast
" 11	63	5,900,000	18,200	18,982	1,162	581	
" 22	70	6,380,000	15,100	8,200	0	0	
" 31	80	5,640,000	17,000	11,400	1,900	0	
1926							
Jan. 12	78	5,900,000	14,000	7,348	668	0	
Feb. 2	73	5,540,000	33,500	8,650	2,422	346	
" 16	89	8,040,000	23,200	7,860	975	245	Killed

magnitude of this reaction varied directly with the size of the dose and the frequency of the administration. The general course of one of the animals given large doses is illustrated in Table I.

In a female which became pregnant, the appearance of nucleated red blood cells in the peripheral blood was markedly depressed for

about 2 weeks, and finally completely suppressed, before the litter was cast, only to be resumed with renewed vigor after the termination of the pregnancy. This observation was verified in another animal allowed to become pregnant during the course of the experiment. In Table II is illustrated the effect of comparatively small dosage, and the influence of pregnancy on the blood picture. It is of interest to note that the appearance of nucleated red blood cells was often concomitant with an increase of the erythrocytes and hemoglobin in healthy animals above the supposedly normal initial level. That the number of nucleated red cells in the peripheral blood represented an actual stimulation of the erythrocytic centers and not a release only of already formed cells in the marrow is evidenced by (1) the length of time that the animals exhibited this phenomenon (one animal 141 days), (2) the microscopic findings of the bone marrow.

The bone marrow in all the animals receiving injections of Weber's Drawing Ink showed a marked hyperplasia. The degree of hyperplasia varied with the length of time of administration and the size of the dose. In those animals receiving large doses for a comparatively short time the tendency seemed to be toward less replacement of fat, while with smaller dosage, the decrease of fat cells was more marked. The hyperplasia was largely limited to cells of the erythrocyte series and normoblasts predominated. When a few small doses were given, younger forms were more evident in the hyperplastic areas. In animals injected over a long period of time, the bone marrow was always hyperplastic in structure, and this hyperplasia included also the leucocyte series. In these cases there was also evidence of a patchy hypoplasia. This was especially marked in No. H-60, in which the patchy hypoplasia was adjacent to areas of most intense erythrocyte hyperplasia. In addition many clasmatocytes, scattered throughout in all the sections, were filled with great quantities of carbon particles, in many instances obscuring the finer architecture of the bone marrow. A striking feature in the bone marrows studied was the absence of the endothelial wall in many of the venous sinusoids. Under low magnification the mature red cells were confined to definite channels, the boundary line of which, under higher magnification, proved to be nucleated red cells in various stages of maturation. Occasionally a large swollen endothelial cell would be in line with a megaloblast or

erythroblast, furnishing part of the boundary line of the blood vessels, but more frequently the boundary consisted of erythroblasts and normoblasts, many of them apparently loose and mixed with mature red cells. This indicates that in these hyperplastic marrows, red blood cell formation was taking place intravascularly, and, as far as could be determined, from the vascular endothelium of patent, functioning sinuses. The same observations have been made by Doan, Cunningham, and Sabin (5).

Other points of interest noted in the microscopic sections were the large number of nucleated red cells in the spleen; the practical absence of carbon particles from the retroperitoneal mesenteric lymph nodes; and the considerable accumulation of carbon particles in the lung. Whether the nucleated red blood cells were taken out of the blood stream or formed locally in the spleen could not be determined.

Injections with Dialysed Weber's Drawing Ink and the Dialysate.

In order to isolate, if possible, the factor producing this stimulation of the erythrocytic centers described above, the ink was dialysed through a parchment membrane in running water. If the process of dialysation was continued more than 16 hours, a gelatinous mass was formed which was unfit for intravenous injections. The results obtained with ink dialysed between 12 and 16 hours gave essentially the same, but less striking results than those obtained with the untreated ink.

The dialysate of the ink, obtained by suspending 100 cc. of the ink in a parchment bag in 50 cc. of distilled water and allowing it to stand for 4 days, when injected intravenously seemed to be without any effect on the blood. The bone marrow of the animal injected was essentially normal.

Injections with Filtrate of Ink.

The filtrate obtained by passing the ink through a Berkefeld filter W gave results similar to the whole ink injected intravenously, and, as far as could be determined, by removing the ink particles the action on the hematopoietic organs was somewhat enhanced. In Table III, the course of one animal so injected is illustrated. The dosage in this animal was 4 cc. of the undiluted filtrate, comparable to an 8 cc. dose

given to Rabbit H-24. In all the animals the initial rise was followed with a marked drop in the hemoglobin and erythrocytes. Both the rise and the anemia following were accompanied by tremendous outpourings of nucleated red cells, the number of which declined somewhat after reaching a maximum. This decline was usually accompanied by a rise in hemoglobin and erythrocyte values. Efforts

TABLE III.

Rabbit H-63. Intravenous Injections of Filtrate of Weber's Drawing Ink. Ink Passed through Berkefeld Filter W. Large Dosage.

Date	Hemoglobin	Red blood cells	White blood cells	Normoblasts	Erythroblasts		Remarks
					With hemoglobin	No hemoglobin	
		per c.mm.					
1925	*per cent*						
Oct. 15	80	6,280,000	14,800	0	0	0	
" 19	79	6,140,000	10,800	0	0	0	
" 20			24,500	0	0	0	Injections begun. Dose 4
" 23	99	6,700,000	23,110	7,590	0	0	cc. three times a wk. White
" 27	96	6,090,000	12,670	6,220	0	0	blood cell count 1 hr. after
" 31	75	5,590,000	9,950	10,240	199	0	first injection
Nov. 5	72	5,890,000	28,920	11,630	290	0	One injection omitted Nov. 6
" 9	57	5,110,000	9,080	5,720	0	0	
" 17	58	4,510,000	7,900	41,310	2,142	0	
" 24	58	4,770,000	15,050	21,190	2,558	0	
Dec. 3	45	3,990,000	16,510	63,630	4,209	0	
" 12	46	3,530,000	24,000	25,540	11,830	258	
" 22	45	3,400,000	20,100	39,760	8,644	864	
" 31	42	3,350,000	21,700	64,900	11,694	1,222	
1926							
Jan. 12	50	2,780,000	14,500	61,230	16,329	1,134	
Feb. 2	52	3,330,000	28,800	41,920	9,600	320	Last injection Feb. 1
" 3	48	3,200,000	21,900	28,930	8,775	325	Killed

were made to exhaust the marrow completely by increased dosage and more frequent administration, but without success. The structure of the marrow was similar to that obtained from animals injected with the whole ink with this difference; that the clasmatocytes filled with ink particles were replaced by enlarged clasmatocytes staining pale red with eosin, usually appearing homogeneous with occasional vacuoles and granular inclusions.

Injections with Higgins' India Ink.

Five animals were injected with Higgins' India Ink, three with the Waterproof Drawing Ink and two with the General Drawing Ink. The dose in all instances was 4 cc. of a 50 per cent suspension, and the number varied between 4 and 34 injections administered every 2nd day. The course of these animals was identical in the main with the results reported by Sapinoso, Berg, and Jobling (6), *i.e.*, first there was a decline of red blood cells and hemoglobin, followed by a rise to the initial level. Nucleated red cells in the normoblastic stage appeared in the peripheral blood, but the magnitude of the reaction was slight when compared with those obtained with Weber's India Ink and its filtrate. At no time were more than 2280 normoblasts found per c.mm. The most striking difference, however, was found in the bone marrow. The carbon was deposited in clasmatocytes to a great extent, but considerable amounts were found in the endothelium lining the dilated blood vessels. There was little or no hyperplasia of the bone marrow and the cells present were mainly of the leucocyte series.

The enormous capacity of the endothelial cells to form clasmatocytes and phagocyte particulate matter is realized when studying sections from animals injected over long periods of time, provided that these particles or colloidal suspensions are of an inert nature. The findings above prove conclusively that carbon particles as such stimulate the endothelial cells mainly toward clasmatocyte formation. With colloidal silver on the other hand, there seems to have been a toxic effect on the endothelial cells incapacitating the entire system very rapidly. This may perhaps explain the divergence of results by investigators "blocking" the so called "reticulo-endothelial system." The colloid or particulate matter used may be inert; it may stimulate or it may depress this widespread system of cells.

Injections with Günther Wagner's India Ink.

Two brands of German manufacture were used, namely, Chin Chin and Pelican Waterproof Ink. The results were essentially the same as with Weber's India Ink, except that the changes of both the peripheral blood and blood-forming organs were less marked.

DISCUSSION.

Summarizing the results obtained by the injection of various India inks, certain features ought to be emphasized. With Weber's India Ink a tremendous outpouring of nucleated red cells occurred which, in the light of the experiments described above, may be attributed to a substance which passes through a Berkefeld filter and seems to be non-dialysable. This substance, apparently a protective colloid, appears to have essentially the same effect on the blood and the blood-forming organs, whether it is associated with the ink or separated from it. With large doses after an initial rise of erythrocyte and hemoglobin values, there was a decrease in these constituents of the peripheral blood, while with smaller dosage there was mainly an increase of red cells and hemoglobin sustained for a variable time and followed later by a decrease. Associated both with the rise and fall in red cells there was an outpouring of nucleated red cells, as a rule proportional to the size of the dose and the number of administrations. The interesting feature, however, is the delivery of the nucleated red cells in the peripheral blood in the face of apparently an adequate number of circulating red cells.

The conditions in which nucleated red cells appear in the peripheral blood were reviewed by Drinker, Drinker, and Kreutzmann (7, *a*) in 1918. Except in anemia due to various causative factors, nucleated red cells do not appear in the peripheral blood save as a result of marrow disintegration (8), as an occasional concomitant of leucocytosis (9, 10), and, very rarely, in animal and man subjected to sudden low barometric pressure (11). The crowding out of the nucleated red cells in acute myelogenous leucemia and in metastatic tumor growth in the marrow (12) is easily explained as pressure phenomena.

With a normal marrow the appearance of nucleated red cells in the peripheral blood stream can in all probability be explained by growth pressure (7, *b*). This pressure may be local and does not necessitate an extensive hypertrophy throughout the tissues. If the picture of the bone marrow from the histological and physiological points of view is kept in mind, the appearance of nucleated red cells in the peripheral blood when the marrow is stimulated becomes apparent. Histologically, we have a tissue with sinuses and intersinusoidal capil-

laries situated between the fat cells, and outside the functional blood stream are the marrow cells, the red cells, however, developing in the intersinusoidal closed, but potential, capillaries as has been shown by Doan, Cunningham, and Sabin (5). Occasionally in a hyperplastic marrow the developing red blood cells may be situated directly in the path of the blood stream as seen in these experiments. Physiologically on the other hand the bone marrow is a highly sensitive tissue confined to an inexpansible environment with all of its elements incompressible, the path of least resistance being the blood stream, as has been pointed out by Drinker (7, *c*).

In the experiments described the appearance of the unprecedented number of normoblasts and erythroblasts in the peripheral blood can therefore be explained by growth pressure. The growth pressure may be produced in this instance by two factors, the most important one being the marked increase of the nucleated red cells in the bone marrow; the other being the number of large clasmatocytes scattered through the bone marrow and encroaching to a considerable extent on the limited space. With this pressure from developing erythrocytes and clasmatocytes the intersinusoidal capillaries may be forced open prematurely, allowing the circulating blood to wash over the immature red cells; or the red cells may develop primarily on the edges of the already open sinuses. Growth pressure due to a considerable increase of the clasmatocytes in the bone marrow may perhaps explain the moderate number of normoblasts found in the peripheral blood of animals injected with Higgins' India Ink, the bone marrow of which showed little or no hyperplasia of the erythrocyte series but a great many clasmatocytes.

As an explanation of the delivery of red cells in the peripheral blood stream, disintegration of the marrow must be thought of, but can be dismissed at once because of the length of time the animals exhibited this phenomenon, the content of the peripheral blood, and the histological picture of the bone marrow. It is of course conceivable that the stickiness of the red cells except the mature erythrocytes, as has been pointed out by Key (13), may be lessened or abolished in some way by the substance used for injections, but this does not explain adequately the phenomena encountered.

The marked suppression of the delivery of nucleated red blood cells

in the blood stream, concomitant with a slight fall in erythrocytes and hemoglobin in the course of pregnancy, I do not attempt to explain, but it is an interesting fact showing the dominant effect of pregnancy on the bone marrow over an agent which otherwise did not fail to elicit this characteristic reaction.

It is evident that from the experiments above no definite conclusions can be drawn as to the nature of this bone marrow stimulus and whether it operates directly on the bone marrow or indirectly through some other organs. Investigation of the subject, however, is now in progress.

CONCLUSIONS.

1. Intravenous injections in rabbits of F. Weber Co.'s Drawing Ink gave the following results:

(*a*) With large doses, an outpouring of nucleated red blood cells, in many instances reaching high values (60,000 to 70,000 per c.mm.), accompanied by a slight initial rise of erythrocytes and hemoglobin, followed by a moderate anemia and in one animal, a severe anemia.

(*b*) With smaller doses the presence of nucleated red cells in the peripheral blood was correspondingly less, the hemoglobin and erythrocytes increasing above the initial level. This increase was sustained for a considerable time, followed by a decrease to or below the initial level.

(*c*) Pregnancy caused a marked suppression, and, shortly before delivery, a complete suppression of the normoblastosis. Nucleated red blood cells reappeared in the blood after the litter was cast.

2. The filtrate of Weber's Drawing Ink, obtained through a Berkefeld filter W, produced the same effects, somewhat enhanced, as the whole ink.

3. The bone marrow of the animals injected with Weber's ink and the filtrate, both showed a marked erythrocytic hyperplasia, with many open sinuses lined with nucleated red blood cells in all stages of maturation.

4. As the dialysed ink gave practically the same results, and the dialysate proved to be without any effect, the conclusion was drawn that a non-dialysable protective colloid was responsible for the marked stimulation of the hematopoietic organs.

5. The delivery of nucleated erythrocytes was interpreted as due to growth pressure induced by rapidly growing red blood cells as well as intrasinusoidal formation of erythrocytes. Pressure due to rapidly increasing phagocytic cells must also be considered.

6. It is fair to conclude that carbon particles as such stimulate endothelial cells mainly toward clasmatocyte formation without incapacitating the endothelial cells, while colloidal silver apparently had a toxic and incapacitating effect on this system of cells.

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LOCAL IMMUNITY IN EXPERIMENTAL ERYSIPELAS.

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Plates 8 to 10.

(Received for publication, November 11, 1926.)

Evidence pointing to the production of a true local tissue immunity following local streptococcus infection has been reported by Gay and Rhodes.[1] These authors used in their experiments on rabbits *Streptococcus pyogenes* ("H") originally obtained from a patient having empyema following an attack of measles. In the experiments to be reported, strains of hemolytic streptococci cultivated from the skin of patients with facial erysipelas have been employed.

In this report, it will be shown that not only the areas of skin actually involved in the reaction attending the immunization are refractory to subsequent injections but that the field in the general direction of the lymph flow is likewise relatively resistant.

Methods.

The strains of beta hemolytic streptococci employed, were isolated from the advancing edge of the erysipelas lesion in human cases by injecting 0.2 cc. of beef infusion broth endodermally and after a half-minute aspirating as much of the injected liquid as possible. The aspirated fluid was usually admixed with blood and a small drop of blood welled up from the track of the needle. These fluids were mixed in the syringe and blown out on rabbit blood agar plates.

The isolated strains were kept on blood agar slants, and from time to time passed through rabbits by endodermal inoculation.

For the endodermal injection, the streptococci were grown on rabbit blood agar slants for 18 hours at 37°C. and washed off in beef infusion broth of pH 7.4. 1/10 of an agar slant growth suspended in 0.25 cc. of broth was the dose usually employed.

The hair over the surface to be injected was removed by clipping closely with sharp curved scissors. Shaving and chemical depilation used in the earlier experiments have been found unsuitable.

[1] Gay, F. P., and Rhodes, B., *J. Infect. Dis.*, 1922, xxxi, 101.

The Experimental Lesion.

The experimental lesion has been described by Rivers[2] who was concerned especially with the diffuse lesion occurring in about two out of ten rabbits injected. In a series of 60 rabbits in which various experiments on local immunity were made, we observed particularly the direction of spread of the lesion from the site of inoculation.

The characteristic redness and edema appeared in all but two animals. In these there seemed to be little defense reaction of the tissues as redness and edema were absent. The skin surrounding the site of inoculation became white and the area of white necrosis spread several cm. eccentrically. Both animals became acutely ill, and after 2 days of high fever, the temperature became subnormal and death occurred on the 3rd day. Hemolytic streptococci were obtained in pure culture from the heart's blood at necropsy.

In five other animals, receiving the usual dose, there was a definite reaction from 1 × 6 cm. to 6 × 6 cm. without the formation of pus; blood invasion and death occurred in from 2 to 7 days.

Pus formation in considerable quantity occurred in nine instances and in only small amounts in nine others. A frequent occurrence was the appearance of small white areas of skin necrosis which on superficial examination suggested pus formation. Such areas measuring from 2 to 10 mm. occurred eighteen times and in five other instances these areas attained a diameter of 6 cm. In three instances in which the Corley strain had been inoculated, the necrotic areas were hemorrhagic. In other instances, not included in these series, Strain B had produced similar lesions.

In general, the lesions may be divided into two classes according to the direction of spread; (1) downward (*i.e.*, ventrally) resulting in a long, narrow band of redness and edema measuring after 48 hours from 1 to 2 cm. in width and from 3 to 17 cm. in length, and (2) circular area of redness and swelling from 1 to 6 cm. in diameter. In the latter, the geometric centers were always below or ventral to the site of inoculation. The relative occurrence of the two types of spread in one series was 28 to 4, in the total number reported here, thirty-nine of Type 1 and nineteen of Type 2. In two instances, there was

[2] Rivers, T. M., *J. Exp. Med.*, 1925, xli, 179.

first a circular spread in which the downward trend met some resistance and after 24 hours there was extension downward resulting in a reniform lesion.

Diffuse localized erythema occurred 4 times among the 60 rabbits on the first injection. In two other rabbits, in which this type of reaction was not present after the first injection, it did occur on one subsequent injection.

Diffuse generalized erythema of the skin has been encountered only twice among 300 rabbits receiving endodermal injection. Both of these rabbits were white without markings.

In the series of 60 rabbits, five rabbits died and showed adhesive pericarditis after several months' immunization.

Lesions Produced by Repeated Injections.

Cobbett and Melsome[3] failed to obtain erysipelas on reinoculation of the same ear of the rabbit during a period of from 22 to 100 days following the first successful inoculation. A second inoculation in the protected ear caused a transitory and limited infection. Gay and Rhodes[1] report protection against endodermal reinoculation enduring from 3 weeks to 3 months in rabbit skin after recovery from experimental erysipelas. These authors used an empyema strain of beta hemolytic streptococcus.

In the course of the production of monovalent immune serum in rabbits by repeated endodermal inoculation, records were kept of the extent of the lesion resulting from the injection of a standard dose at weekly intervals.

Whereas the spread after the first endodermal injection more often descended ventrally in the direction of the lymph flow rather than anteroposteriorly, after the second subsequent injections the resulting lesions were usually circular and eccentric to the point of injection. The circular lesions resulting from the first injection were compared with the same type following the subsequent inoculations in seventeen rabbits. The average maximum diameter after the first injection was 2.2 cm., after the second, 2.0 cm., the third, 1.7 cm., and the fourth, 0.8 cm.

[3] Cobbett, L., and Melsome, W. S., *J. Path. and Bact.*, 1896, iii, 39; *Centr. allg. Path. u. path. Anat.*, 1898, ix, 827.

Increased Resistance of the Skin on One Side.

In the course of immunization with various strains by the endodermal injection of living streptococci, some of the rabbits were by chance injected several times at intervals on only one side. After several injections, the reactions to which were successively smaller, an injection was made on the other side of the rabbit, with the result that the lesion was practically as large as after the first immunizing dose. The protocols of six experiments illustrating the difference in the response of the non-injected and the injected sides in partially immunized rabbits are shown below.

Experiment 1.

Rabbit 1.—Oct. 30, 1925. Hair removed from left side by clipping with scissors followed by shaving. Into two sites in depilated area was injected, endodermally, 0.4 cc. of broth containing 4/10 of the 18 hour growth of Strain Z on a rabbit blood agar slant. The resulting lesions were red and edematous, the larger of which was 1 × 5 cm. in extent.

Nov. 19. Hair removed from both sides. At two points in each depilated area there was injected 0.25 cc. of broth containing 2/10 of the 18 hour growth of Strain Z on a rabbit blood agar slant. The resulting lesions were 2 cm. in diameter on the left side and 3.5 cm. on the right.

Rabbit 2.—Oct. 3, 1925. Hair removed from left side by clipping with scissors, followed by shaving. Into the depilated area there were injected endodermally, (1) 0.4 cc. of broth containing 4/10 of the 18 hour growth of Strain Z on a rabbit blood agar slant, and (2) 0.8 cc. containing 8/10 of a similar culture of Strain C. Reactions consisted in edema and reddening 3 × 1.5 cm. with Strain Z, and 6 × 4 cm. with Strain C.

Oct. 30. Left side injected at one point with 0.5 cc. of broth containing 1/2 of the 18 hour growth of Strain C on a rabbit blood agar slant. Resulting lesion consisted in edema and reddening 3 × 5 cm. in area.

Nov. 11. Left side injected with 1 cc. containing 7/10 of the 18 hour growth of Strain C on a rabbit blood agar slant. Resulting lesion, measuring 1.5 × 3 cm., was very red and slightly elevated and indurated.

Nov. 19. Hair removed from both sides. Each side received an injection of 0.4 cc. of broth containing 4/10 of the 18 hour growth of Strain C on a rabbit blood agar slant. Lesion: on left was 2.5 cm., on right, 5 cm. in diameter.

Rabbit 3.—Oct. 3, 1925. Hair removed from left side by clipping with scissors, followed by shaving. Into depilated area there was injected endodermally 0.4 cc. of broth containing 4/10 of the 18 hour growth on a rabbit blood agar slant of Strain P. The resulting lesion, measuring 3 cm. in diameter, was red and edematous.

Oct. 30. 0.5 cc. of broth containing 1/2 of the 18 hour growth of Strain P on a rabbit blood agar slant was injected endodermally on the same side. The resulting lesion was 2 cm. in diameter.

Nov. 19. There was injected endodermally on both sides 0.2 cc. of broth containing 2/10 of the 18 hour growth of Strain P on a rabbit blood agar slant. Resulting lesion, left side, slight reddening and edema 1 cm. in diameter; right side, marked edema and reddening 3 cm. in diameter.

Rabbit 4.—Sept. 28, 1925. Hair removed from left side by clipping followed by shaving. Into the depilated area, there was injected endodermally 0.5 cc. of broth containing 1/2 of the 18 hour growth of Strain P on a rabbit blood agar slant. Result: induration and reddening 1 cm. in diameter.

Nov. 12. 0.25 cc. of broth containing 1/4 the 18 hour growth of Strain P on a rabbit blood agar slant was injected endodermally. There was slight reddening and edema, after 24 hours.

Nov. 19. 0.2 cc. of broth containing 2/10 of the 18 hour growth of Strain P on a rabbit blood agar slant was injected endodermally on each side.

Resulting lesion on left side, slightly reddened papule 0.5 cm. in diameter; right side, intense reddening 2 cm. in diameter.

Rabbit 5.—Sept. 28, 1925. Hair removed from left side by clipping with scissors followed by shaving. Into the depilated area, there were injected endodermally (1) 0.5 cc. of broth containing 1/2 of the 18 hour growth of Strain B on a rabbit blood agar slant and (2) an equal dose of Strain Z. Resulting lesions from Strain B, reddening and edema 3.5 × 1.5 cm.; from Strain Z, reddening and edema 1 cm. in diameter.

Nov. 12. 0.4 cc. of broth containing 4/10 of the 18 hour growth of Strain B on a rabbit blood agar slant was injected endodermally at two points on left side. Resulting lesions, reddening and edema 1 × 3 and 3 × 3 cm.

Nov. 19. 0.2 cc. of broth containing 2/10 of the 18 hour growth of Strain B on a rabbit blood agar slant was injected at one point on each side. Resulting lesion, left side, reddening and edema 1.5 cm. in diameter; right side, 4.5 cm.

Experiment 2.

Rabbit 6.—Sept. 28, 1925. Hair removed from left side by clipping followed by shaving. Into the depilated area there was injected endodermally 0.5 cc. of broth containing 1/2 of the 24 hour growth of Strain H on a rabbit blood agar slant. Resulting lesion: reddening and induration 2 cm. in diameter.

Nov. 11. 0.4 cc. of broth containing 4/10 of the 18 hour growth of Strain H on a rabbit blood agar slant was injected endodermally on the left side. Resulting lesion: induration and intense reddening with skin necrosis 2 cm. in diameter.

Nov. 21. 0.25 cc. of broth containing 1/4 of the 18 hour growth of Strain H on a rabbit blood agar slant was injected endodermally at two points on each side. Resulting lesions, left, reddening and edema 1 cm. in diameter; right, reddening and edema 1.5 and 3 cm. in diameter.

Extent of Local Immune Area.

The skin previously invaded by the erysipelas lesion is markedly resistant to subsequent infection with strains of the erysipelas group.

Since the spread of the lesion is usually downward, or ventral to the point of injection, it is possible that areas in the direction of the lymph flow may also become resistant even though such areas may not be visibly involved in the erysipelas lesion. To test this possibility, injections of the same dose were made on the same day at various points into the skin surrounding the healed area in partially immunized rabbits. The points of injection were usually (1) ventral to the edge of the previous area involved, (2) anterior or proximal, and (3) on the other side of the rabbit where no previous injections had been made.

The results of these injections show definitely that areas in the direction of the lymph flow though not within the previously involved area are refractory to the injections of the homologous strains. The protocols follow.

Experiment 3.

Rabbit 5.—Immunized against Strain B. Injections begun Sept. 28, 1925, and repeated once a week.

Dec. 7. Injected endodermally with 0.2 cc. containing 2/10 of the 18 hour growth of Strain B on a rabbit blood agar slant at each of the following points equidistant from the old lesion: (1) ventral to the site of the old lesion; (2) dorsal to it; (3) anterior to it.

Resulting lesions were respectively, 1.5 cm. in diameter, 3 × 2 cm., and 2. 5 × 0.5 cm.

Rabbit 2.—This rabbit had received four endodermal injections of 4/10 of the culture of Strain C, well separated on the left side at weekly intervals during the preceding 4 weeks. The last injection was on Dec. 7, 1925. On Dec. 14, three endodermal injections each of 4/10 of a culture of Strain C in 0.4 cc. broth were made at three different points:

(1) 1 cm. below the edge of the lesion of Dec. 7; (2) 1 cm. anterior to the same lesion; and (3) at a corresponding point on the right side.

The maximum measurements at the three sites were 1 cm., 2.5 cm., and 5 cm., respectively. Photographs of these lesions are shown in Figs. 1 and 2.

Experiment 4.

Rabbit 7.—Dec. 1, 1925. Hair removed from left side by close clipping. Into two sites in depilated area was injected endodermally 0.25 cc. of broth containing

2/10 of the 18 hour growth of Strain B on a rabbit blood agar slant. Resulting lesions measuring 2 × 4 and 2 × 5 cm. after 24 hours were red and edematous with small central patches of skin necrosis.

Jan. 29, 1926. The same dose of living organisms (Strain B) was injected endodermally at two points on the left side, one below site of old lesions and the other adjacent to it, and at two points on the right side. Resulting lesions, left side, below site of first inoculation, a small red swelling 0.5 × 2 cm. in area, with a patch of skin necrosis at center; adjacent lesion, red and edematous, 1.5 × 3 cm. Right side, reddening and edema and slight skin necrosis 1.5 and 3 cm. in diameter.

Rabbit 8.—Injected on same dates and with same doses as Rabbit 7, but the organism used was Strain C. First injections on left resulted in two areas of reddening and edema, 3 × 5 cm. in size. After the second series of injections, the lesion below the old site of inoculation consisted in a red papule 1 cm. in diameter. Adjacent to it, the lesion was 1 × 3 cm. in size and showed very little swelling. On the other side, the lesions consisted in reddening and edema 2 × 3 cm. in area.

Experiment 5.

Rabbits 9, 10, 11 } Apr. 15, 1926. Hair removed from left side by close clipping with scissors. Into depilated area was injected endodermally 0.2 cc. of broth containing 1/500 of the 18 hour growth on a rabbit blood agar slant of the following strains:

Rabbit 9 received Strain P; Rabbit 10, Strain B; Rabbit 11, Strain C.

Resulting lesions after 24 hours: Rabbit 9, reddening and intense edema 1.5 × 2 cm.

Rabbit 10: Edema and reddening 2 × 3.5 cm.

Rabbit 11: Edema, reddening, and large patch of skin necrosis 2 × 6.5 cm.

May 22. Hair removed from both sides by clipping. The three rabbits were injected with their respective strains at four points with 0.2 cc. of broth containing 1/500 of the 18 hour growth on a rabbit blood agar slant, as follows:

1. Left side: Below old lesion.
2. " " On site of old lesion.
3. " " To the side of old lesion.
4. Right side.

Resulting lesions after 24 hours:

Rabbit 9: left side, below old lesion, edema and reddening 1.5 cm. in diameter.

On site of old lesion, slight edema and reddening 0.8 cm. in diameter. To the side of old lesion, edema and reddening 1.8 cm. in diameter. Right side, edema and reddening 2 × 2.3 cm. in diameter (Figs. 3 and 4).

Rabbit 10: Left side, below old lesion, edema and reddening 2 × 1.8 cm.

On site of old lesion, slight edema and reddening, 0.6 × 0.5 cm.

To the side of old lesion (approximately 5 cm. distant) marked edema and reddening 2.3 cm. in diameter.

Right side, marked edema and reddening 3.5 × 4 cm. (Figs. 5 and 6).

Rabbit 11: Left side, below old lesion, slight edema and very slight reddening 1 × 1.5 cm.

On site of old lesion, slight edema, no reddening, 0.8 × 1.3 cm.

To the side of old lesion, edema and reddening 1.8 × 2 cm.

Right side, reddening and marked edema 2 × 2.3 cm.

The point of greatest resistance to a subsequent infection is shown to be the site of the old lesion; an area ventral to the old lesion is more resistant than one the same distance away on the side or dorsal to it. The uninoculated side remains susceptible.

Local Skin Resistance Induced by Heterologous Erysipelas Strain.

Thus far, the homologous strains have been used in testing the relative resistance to subsequent injections. To determine the effect of injections of a heterologous strain, rabbits were immunized against Strain B and others against Strain P, both representative beta hemolytic streptococci from cases of human erysipelas but differing immunologically as shown by their failure to cross-agglutinate.

When the rabbits had become locally immune against the homologous strain, endodermal injections of both strains were made into each rabbit and into a normal control.

Sample protocols are given below.

Experiment 6.

Rabbit 12.—Immunized against Strain B. Injections started Oct. 3, 1925, and repeated once a week for 9 weeks; after lapse of 2 months, recommenced Feb. 4, 1926, and continued until Apr. 7.

Apr. 7. Injected endodermally with 0.2 cc. of broth containing 1/10 of the 18 hour growth of Strain B on a rabbit blood agar slant, and with an equal dose of Strain P. Resulting lesion, Strain B: erythema 2 cm. in diameter with small central nodule 0.8 cm. in diameter; Strain P: erythema 3 cm. in diameter, central nodule 0.4 cm. (Fig. 7).

Rabbit 3.—Immunized against Strain P over same period as Rabbit 12.

Apr. 7, 1926. Injected endodermally with Strains B and P in same doses as Rabbit 12. Resulting lesions, Strain B, erythema 2.5 cm. in diameter, slight induration; Strain P, slight erythema and induration 1 cm. in diameter (Fig. 8).

Rabbit 13.—No previous injections. Control for Rabbits 12 and 3.

Apr. 7, 1926. Injected endodermally with Strains B and P in same doses as Rabbits 12 and 3. Both resulting lesions 2 × 10 cm. indurated and reddened with small patches of skin necrosis at points of inoculation (Fig. 9).

Rabbit 14.—June 5, 1926. Injected endodermally on right side 0.2 cc. of broth containing 1/500 of the 18 hour growth of Strain B on a rabbit blood agar slant. Resulting lesion 2.8 × 1.7 cm., edema and reddening with small patch of skin necrosis. There was also a generalized erythema.

July 3. Injected endodermally with 0.1 cc. of broth containing 1/500 of the 18 hour growth of Strain C on a rabbit blood agar slant. Injections made at the following places: (1) on old lesion; (2) below old lesion; (3) on same side, 3 cm. away; (4) on the other side.

Resulting lesions, (1) on old lesion, very small hard red nodule 0.3 cm. in diameter; (2) below old lesion, markedly red and edematous, 1.5 × 0.7 cm.; (3) to the side of old lesion, reddening and edema 1.5 × 1.9 cm., with very small patch of skin necrosis; (4) on the other side, reddening and edema 1.5 × 1.6 cm. with very small patch of skin necrosis.

Rabbit 15.—June 5, 1926. Injected endodermally on the right side with 0.2 cc. containing 1/500 of the 18 hour growth of Strain P on a rabbit blood agar slant. Resulting lesion, slight edema, 1.8 × 6.2 cm.; practically no reddening; extensive skin necrosis, 1 × 3 cm.

July 3. Injected endodermally 0.1 cc. of broth containing 1/500 of the 18 hour growth of Strain B on a rabbit blood agar slant. Injections made at the following places: (1) on site of old lesion; (2) below old lesion; (3) on same side, 3 cm. away; (4) on the other side.

Resulting lesions, (1) on old site, very small hard red papule, 0.3 cm. in diameter; (2) below old lesion, barely palpable, faint reddening 1 cm. in diameter; (3) to the side of old lesion, barely palpable slight reddening 1.5 cm. in diameter; (4) on the other side, distinct edema and reddening 1.5 × 2 cm.

Rabbit 16.—June 5, 1926. Injected endodermally on the right side 0.2 cc. of broth containing 1/500 of the growth of Strain C on a rabbit blood agar slant. Resulting lesion 2.5 × 2.8 cm., marked edema and reddening.

July 3. Injected endodermally 0.1 cc. of broth containing 1/500 of the 18 hour growth of Strain B on a rabbit blood agar slant. Injections made at the following places: (1) on site of old lesion; (2) below old lesion; (3) on same side, 3 cm. away; (4) on the other side.

Resulting lesions, (1) on old lesion, faint reddening and very slight edema 1 × 1.5 cm.; (2) below old lesion, faint reddening and very slight edema 1.8 cm. in diameter; (3) to the side of old lesion, edema barely palpable, reddening very faint 0.8 cm. in diameter; (4) on the other side, slight reddening and very slight edema 1 × 1.7 cm.

The experiments show very definitely that one member of the erysipelas group of streptococci is capable of stimulating local skin resistance against a second member of the group which is immunologically different from the first. This offers some hope of attaining a polyvalent anti-erysipelas serum which is so desirable and attests to

the close relation of members of this group even though their agglutination reactions do not establish a complete identity.

Local Resistance to Other Hemolytic Streptococci.

Rabbits immunized to Erysipelas Strains B, P, and H were tested against a strain of beta hemolytic streptococcus isolated from the tonsils during the acute stage of follicular tonsillitis.

The test tonsillitis strain, Heyder, was passed through three rabbits to insure constant virulence and to ascertain the infecting dose in the normal rabbit skin.

The results of this experiment are shown in the following protocols.

Experiment 7.

Rabbit 12.—Immunized against Strain B. Injections started Oct. 3, 1925, and repeated once a week for 9 weeks; after lapse of 2 months, recommenced Feb. 4, 1926, and continued until June 2.

June 2. Injected endodermally with 0.1 cc. of broth containing 1/20 of the 18 hour growth of Strain B on a rabbit blood agar slant, and with 1/10 of the 18 hour growth of the Heyder strain on a rabbit blood agar slant. Resulting lesions after 48 hours, Strain B, faint reddening 0.9 cm. in diameter, small elevation 0.3 cm. in diameter; Heyder strain, moderate edema and reddening 3 × 3.2 cm.

Rabbit 3.—Immunized against Strain P over same period as Rabbit 12.

June 2, 1926. Injected endodermally with Strain B and the Heyder strain in same doses as Rabbit 12. Resulting lesions, Strain B, reddening and edema 2.1 × 1.3 cm.; tonsillitis strain, edema and very slight reddening 2 × 2.5 cm.

Rabbit 6.—Immunized against Strain H. First injection Sept. 28, 1925, second, Nov. 11, repeated once a week until Dec. 27. Recommenced Jan. 29, 1926, and continued until June 2.

June 2. Injected endodermally with Strain B and the Heyder strain in same doses as Rabbits 12 and 3. Resulting lesions, Strain B, slight edema and reddening 1.2 × 1.8 cm.; Heyder strain, edema and reddening, 3.2 × 1.5 cm.

Rabbit 17.—No previous injections. Control for Rabbits 12, 3, and 6, in this experiment.

June 2, 1926. Injected endodermally with Strain B and the Heyder strain in same doses as Rabbits 12, 3, and 6. Resulting lesions, Strain B, edema and reddening, 2.0 × 1.8 cm., with large patch of skin necrosis; Heyder strain, slight edema and reddening, 1.5 × 4.5 cm., with small patch of skin necrosis.

Experiment 8.

Rabbit 18.—June 2, 1926. Injected endodermally on right side 0.1 cc. of broth containing 1/500 of the 18 hour growth of Strain B on a rabbit blood agar slant.

Resulting lesion, 1.8 × 2.3 cm., reddening and edema with small central patch of skin necrosis.

July 2. Injected endodermally 0.1 cc. of broth containing 1/20 of the growth of the Heyder strain on a rabbit blood agar slant 24 hours old. Injections made at the following places: (1) on old lesion; (2) below it; (3) on the same side, 3 cm. away; (4) on the other side.

Resulting lesions, (1) on old lesion, small red papule 0.5 × 0.8 cm.; (2) below old lesion, slight reddening and edema 1.5 × 1.8 cm.; (3) to the side of old lesion, reddening and edema 1 cm. in diameter; (4) on the other side edema and reddening 1.5 × 3.5 cm. with a purplish hemorrhagic centre (Figs. 10 and 11).

These tests show that the skin of a rabbit immunized against a strain of beta hemolytic streptococcus from erysipelas possesses considerable resistance to beta hemolytic streptococci from another source, but less than that exhibited against the erysipelas strain.

Local Resistance to Staphylococcus aureus.

Rabbits immunized to Erysipelas Strains B, P, and H were given endodermal injections of test doses of a culture of *Staphylococcus aureus* isolated from a human case of staphylococcemia following cellulitis. The test strain had been passed through five rabbits and was found to produce a marked reaction when injected into the skin of the normal rabbit. The results of these tests are shown in the following protocol.

Experiment 9.

Rabbit 12.—Immunized against Strain B. Injections started Oct. 3, 1925, and repeated once a week for 9 weeks; after lapse of 2 months, recommenced Feb. 4, 1926, and continued until June 2.

June 2. Injected endodermally 0.1 cc. of broth containing 1/20 of the 18 hour growth of Strain B on a rabbit blood agar slant and with 1/20 of the 18 hour growth of a *Staphylococcus aureus* strain on a 7.4 beef infusion agar slant. Resulting lesions after 48 hours; Strain B, faint reddening 0.9 cm. in diameter, small elevation 0.3 cm. in diameter; staphylococcus strain, after 48 hours, slight reddening 1 cm. in diameter, small yellowish elevation 0.3 cm. in diameter.

Rabbit 3.—Immunized against Strain P over same period as Rabbit 12.

June 2, 1926. Injected endodermally with Strain B and the staphylococcus strain in same doses as Rabbit 12. Resulting lesions, Strain B, reddening and edema 2.1 × 1.3 cm.; staphylococcus strain, reddening and edema 1.8 × 0.8 cm.

Rabbit 6.—Immunized against Strain H. First injection Sept. 28, 1925, second on Nov. 11, repeated once a week until Dec. 27. Recommenced Jan. 29, 1926, and continued until June 2.

June 2. Injected endodermally with Strain B and the staphylococcus strain in same doses as Rabbits 12 and 3. Resulting lesions, Strain B, slight edema and reddening 1.2 × 1.8 cm.; staphylococcus strain, edema and reddening 1.3 × 1.8 cm.

Rabbit 17.—No previous injections. Control for Rabbits 12, 3, and 6 in this experiment.

June 2. Injected endodermally with Strain B and the staphylococcus strain in same doses as Rabbits 12, 3, and 6. Resulting lesions, Strain B, edema and reddening, 2.0 × 1.8 cm., with large patch of skin necrosis; staphylococcus strain, edema and reddening, 2 cm. in diameter, with small patch of skin necrosis.

It appears from these experiments that the skin of rabbits immunized against beta hemolytic streptococci from erysipelas is also slightly resistant to infection by *Staphylococcus aureus.*

Resistance to Staphylococci of Contiguous Areas.

To test the spread of local resistance to staphylococci from an area previously injected with streptococci, an endodermal injection of Hemolytic Streptococcus C was made on the left side of a rabbit and after an interval of 30 days the contiguous areas were inoculated with the staphylococcus. Details of the experiment are shown in the following protocol.

Experiment 10.

Rabbit 19.—June 2, 1926. Injected endodermally on right side with 0.1 cc. of broth containing 1/500 of the 18 hour growth of Strain C on a rabbit blood agar slant. Resulting lesion, reddening and marked edema, 1.7 × 1.8 cm., with narrow zone of hemorrhage round the edge.

July 2. Injected endodermally 0.1 cc. of broth containing 1/20 of the growth on a 7.4 beef infusion agar slant of the virulent strain of *Staphylococcus aureus.* Injections made at the following places: (1) on old lesion; (2) below old lesion; (3) on the same side, 3 cm. away; (4) on the other side (Figs. 12 and 13).

Resulting lesions, (1) on the old lesion, reddening and marked edema, 0.8 × 1.6 cm., with tiny patch of skin necrosis; (2) below old lesion, reddening and marked edema, 1.8 × 2.8 cm., with small patch of skin necrosis; (3) to the side of old lesion, reddening and marked edema, 2.3 × 3.5 cm., with small patch of skin necrosis; (4) on the other side, reddening and marked edema, 2 × 2.3 cm., with small patch of skin necrosis.

Thus, 30 days after an injection of streptococci, the area of skin involved in the inflammatory reaction was somewhat refractory to an injection of virulent staphylococci. But the difference between the

lesion on the treated and that produced on the other side of the rabbit was not so great as in the experiments in which the same strain of streptococci was employed both for the local immunizing inoculation and the subsequent tests.

As in the experiment in which the same strain of streptococci was used the area ventral to the site of previous injection was refractory to subsequent injection but the non-specific resistance was not as great as the specific resistance. The endodermal injection of staphylococci anterior to the area involved in the immunizing process resulted in a lesion somewhat larger than that on the other side of the rabbit where no previous injection had been made. This result is in accordance with the experiment in which the homologous strain was used to test the direction of the spread of the local immune area.

Repeated endodermal injections induce a gradual spread in the areas of increased resistance until general immunity is established. After the lesion produced by the original inoculation has subsided, usually in 2 or 3 weeks, the skin which had been involved is highly resistant to subsequent infection. The contiguous areas are slightly resistant and the regions in the assumed direction of the lymph flow still more refractory to subsequent infection. At this time the skin on the other side of the rabbit has not acquired any resistance. As the immunization proceeds by weekly injections on one side, the opposite side which has never been injected begins to show resistance. But, at the same time, there is evidence of humoral immunity as shown by the presence of agglutinins and antitoxic properties in the blood serum.

There are differences not shown in the averages. As the weekly injections were made, the redness and edema diminished but no records were kept of the extent to which the lesions were raised above the surrounding tissues. There are, of course, exceptions to the averages, for example, in one instance the lesion after the first injection measured 3 cm. and after the second, 5 cm., yet after the third and fourth, 0.6 and 0.2 cm. respectively.

In the course of repeated injections when the lesions produced by the standard dose decreased progressively in size, the reaction within an hour after injection was sometimes surprisingly large and was characterized by great edema rather than redness, and by abrupt subsidence. The succeeding injections did not regularly show this

phenomenon. The same observations have been made in horses that were undergoing immunization by the endodermal method. The question of an allergic response will be discussed in later publications.

These results suggest the gradual development of skin resistance to repeated endodermal inoculation.

SUMMARY.

The experiments recorded show that the skin involved in the zone of inflammation produced by the endodermal injection of virulent living erysipelas streptococci into rabbits becomes, after the lesion has healed, partially but not completely resistant to subsequent infection with the homologous organism. In the majority of experiments the lesion resulting from the first endodermal injection spread downward, *i.e.*, ventrally, to the site of needle puncture forming an oval or elongated inflammatory zone. Subsequent injections of the same strain into the skin beyond and ventral to the apparent edge of the lesion showed that these areas in the supposed direction of the lymph flow likewise became resistant but not quite to the same degree as in the inflammatory area. The contiguous areas above, behind, and in front of the healed lesion exhibited only a very mild degree of resistance. The other side of the rabbit where no previous injections had been made reacted as did the normal skin.

Repeated injections on the same side bring about diminishing local reaction until there is almost no lesion following the injection of the standard skin dose. Thus there is a gradual spread of skin resistance on the inoculated side, whereas the non-injected side of the rabbit reacts normally. Finally, however, after many injections over relatively long periods the non-injected side becomes resistant, but at this time, there is evidence of general humoral immunity as shown by the presence of agglutinins and antitoxin in the blood. The local resistance is apparently not entirely specific, for the areas with the previous lesion and ventral to it become more resistant to another strain of beta hemolytic streptococcus, though in less degree, and to a virulent strain of *Staphylococcus aureus* to a still less degree. The most plausible explanation of the spread of the local immunity ventrally is that the streptococci follow the lymph channels. Indeed, in human

erysipelas, the organisms are recovered by cutipuncture as far as 3 cm. beyond the advancing edge of the lesion where there is no gross evidence of inflammation. This aspect of local immunity will be considered in subsequent publications.

The skin of the rabbit involved in the inflammatory reaction following the endodermal injection of living streptococci becomes resistant to subsequent injection of the homologous strain and of other strains of erysipelas streptococci which are not immunologically identical.

The local immune areas are resistant but not to the same degree to a strain of hemolytic streptococci isolated from follicular tonsillitis and to a virulent *Staphylococcus aureus*.

The areas contiguous to the local lesion but outside the apparent boundary of inflammation become more resistant to subsequent injection. But the regions ventral to those areas become more resistant than those dorsal, anterior, or posterior to the inflammatory zone.

The skin on the non-injected side of the rabbit becomes resistant *pari passu* with the development of humoral immunity.

EXPLANATION OF PLATES.

PLATE 8.

FIG. 1. Rabbit 2—Experiment 3. Lesions on left side resulting from injections below site of a previous inoculation and adjacent to it. The white spot at the lower edge of shaved area and below a point of inoculation is due to complete depilation and not edema.

FIG. 2. Same rabbit. Lesion on right side which had received no previous injection.

FIG. 3. Rabbit 9—Experiment 5. Lesions on left side resulting from injections on the site of an old lesion, below it, and adjacent to it.

FIG. 4. Same rabbit. Lesion on right side which had received no previous injection.

FIG. 5. Rabbit 10—Experiment 5. Lesions on left side resulting from injections on the site of an old lesion (*b*), below it (*c*), and anterior to it (*d*). The point of original inoculation is *a*.

FIG. 6. Same rabbit. Lesion on right side which had received no previous injection.

PLATE 9.

FIG. 7. Rabbit 12—Experiment 6. Immunized against Strain B. Lesion at left produced by Strain B; that at right, by Strain P.

FIG. 8. Rabbit 3. Immunized against Strain P. Lesion at left produced by Strain B; that at right, by Strain P.

FIG. 9. Rabbit 13. No previous injections. Control for Rabbits 12 and 3 in above experiment.

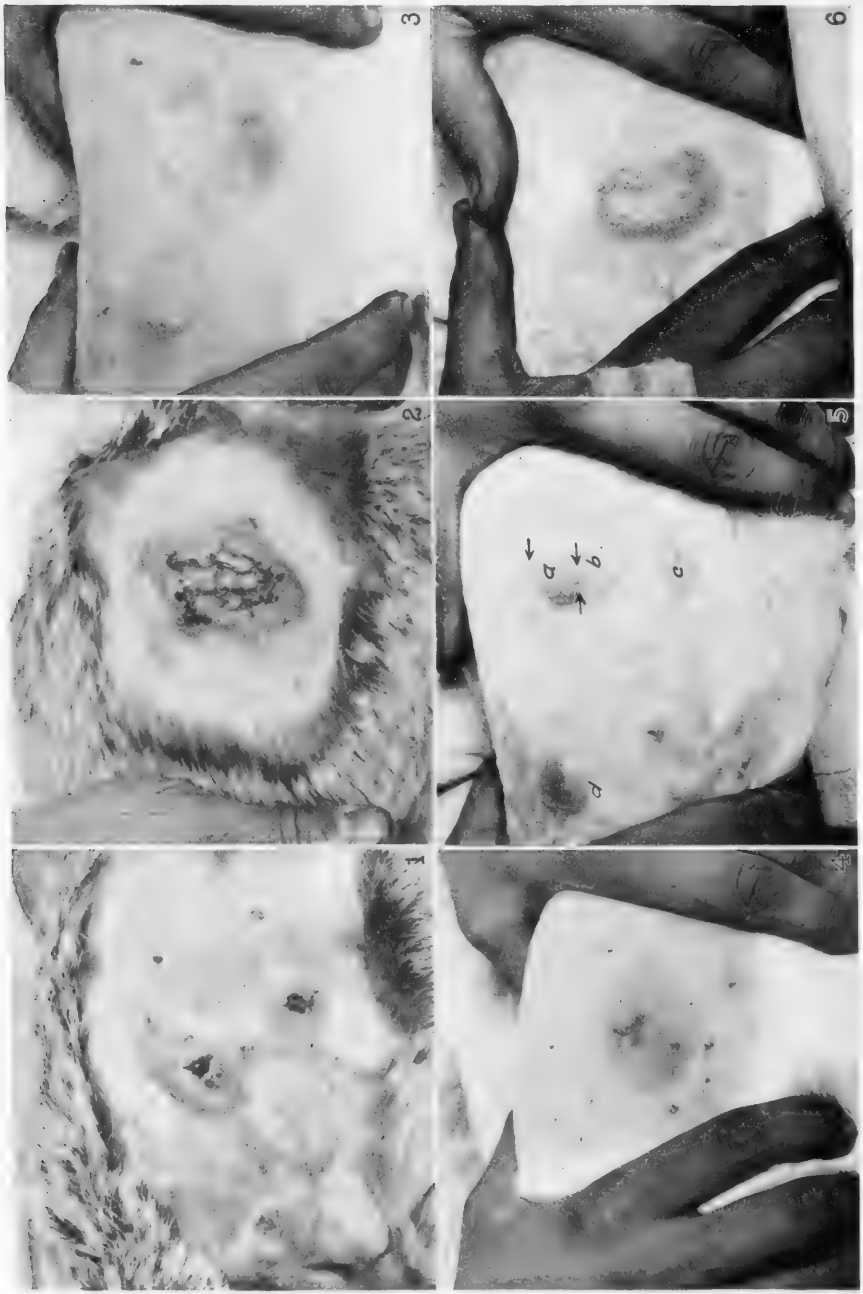
PLATE 10.

FIG. 10. Rabbit 18—Experiment 8. Lesions on right side resulting from injections with a tonsillitis strain on the site of a previous inoculation with an erysipelas strain, below the site and posterior to it.

FIG. 11. Same rabbit. Left side which had received no previous injection.

FIG. 12. Rabbit 19—Experiment 10. Lesions on right side resulting from injections with a strain of *Staphylococcus aureus* on, below, and posterior to the site of a previous inoculation with an erysipelas strain.

FIG. 13. Same rabbit. Left side which had received no previous injection.



(Amoss and Eliss: Local immunity in experimental erysipelas.)

(Amoss and Bliss: Local immunity in experimental erysipelas.)

(Amoss and Bliss: Local immunity in experimental erysipelas.)

BLOOD FIBRIN IN UPPER GASTROINTESTINAL TRACT OBSTRUCTION.

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Foster and Whipple (1–4) have studied the blood fibrin in a number of experimental conditions. They have shown that fibrin is a very labile protein with wide fluctuations due to a variety of stimuli. The site of production is mainly the liver where there is a constant manufacture to supply the continuous utilization in the animal body. Injury to any tissue except the liver causes some increase in the fibrin content of the blood. If the liver is seriously injured a decrease is observed. With mild liver injury there is a rise instead of a fall.

Dead or injured tissue and non-specific inflammation seem the only factors needed to excite an increased production. It seems quite probable also that any cell injury even without inflammatory reaction will cause this characteristic rise. McLester (5) has attempted to utilize blood fibrin determinations as an index of liver function. Foster (6) made fibrin determinations in numerous clinical conditions. He found a marked elevation with the toxemia of pregnancy, the fibrin level running parallel with the severity of the symptoms. With nephritis no increase was observed. Liver atrophy showed a very low value and lobar pneumonia a very high value.

We have made a series of plasma fibrin determinations for comparison with data previously observed in the toxemia incident to upper gastrointestinal tract obstruction.

Methods.

All experiments were done on dogs. All operations were done under anesthesia with aseptic technique. The cardiac end of the stomach and pylorus were obstructed by ligation with tape. The jejunum was obstructed by severing the intestine and turning in the cut ends. The hematocrit reading was obtained by centrifuging 10 cc. of blood mixed with 2 cc. of 1.6 per cent sodium oxalate. The

fibrin determinations were made on this oxalated plasma by the method of Foster and Whipple (1). The non-protein nitrogen was determined by the method of Folin and Wu (7) and the chloride after the manner suggested by Gettler (8).

TABLE I.

Obstruction of Cardiac End of Stomach.

Dog No.	Day after operation	Hematocrit reading (per cent of cells)	Fibrin (mg. per 100 cc. plasma)	Blood (mg. per 100 cc.)	
				Non-protein nitrogen	Chlorides (as NaCl)
		per cent	*mg.*	*mg.*	*mg.*
11	0	55	605	47.6	530
	1	51	980	46.0	490
	2	51	1350	65.5	480
	3	54	1717	101.0	470
	4	43	1608	106.0	570
4	0	50	569	44.4	520
	1	56	1020	60.0	470
	2	61	1210	45.6	440
	3	52	1468	54.5	420
	4	50	1554	118.0	440
	5	55	1600	163.0	490
7	0	50	572	50.5	520
	1	63	972	28.9	490
	2	63	1006	78.0	500
	3	64	1414	130.0	520
8	0	58	665	28.9	470
	1	64	1170	31.9	450
	2	67	1679	82.5	440
	3	61	1828	139.0	400
12	0	54	520	37.3	480
	1	62	975	140.0	410
	2	60	1122	152.0	380

EXPERIMENTAL OBSERVATIONS.

Successive determinations of plasma fibrin after obstruction of the cardiac end of the stomach are shown in Table I. In each animal there is a marked increase, the fibrin being almost tripled. The fibrin

increase parallels quite closely the degree of toxemia as indicated by the level of the non-protein nitrogen of the blood.

TABLE II.

Obstruction of Pylorus.

Dog No.	Day after operation	Hematocrit reading (per cent of cells)	Fibrin (mg. per 100 cc. plasma)	Blood (mg. per 100 cc.)		Remarks
				Non-protein nitrogen	Chlorides (as NaCl)	
		per cent	*mg.*	*mg.*	*mg.*	
13	0	53	368	32.6	480	
	2	61	564	67.5	460	
	3	53	1721	139.0	440	
14	0	46	388	38.9	390	
	1	53	926	36.6	—	
	2	54	840	48.7	450	
	4	61	1212	167.0	420	
10	0	45	566	33.0	470	
	2	60	1380	68.3	420	Obstruction released
	4	48	1301	113.0	320	
	5	42	1148	91.0	440	
15	0	53	456	59.7	490	
	1	57	513	75.0	400	
	2	60	1326	70.0	360	" "
	3	50	1348	107.0	330	
9	0	66	324	30.0	490	
	1	64	1109	30.3	420	
	2	64	1241	59.5	330	" "
	3	58	1036	41.6	390	
	4	58	1062	41.0	380	
	5	69	766	32.6	380	
	6	57	756	38.9	390	
	7	45	751	31.9	400	
	8	43	719	27.0	400	
	9	40	677	35.3	390	
	10	42	845	32.6	420	

With obstructions of the pylorus (Table II) the findings are quite similar. The increase here is even more marked and rapid. In three

animals the obstruction was released after onset of the toxemia. In two the fibrin level remained high and the animals died. One recovered. In this animal the fibrin fell but did not return to the normal level. In four dogs with the jejunum obstructed there was also an increase in the fibrin level. This was not so marked however as with

TABLE III.

Obstruction of Jejunum.

Dog No.	Day after operation	Hematocrit reading (per cent of cells)	Fibrin (mg. per 100 cc. plasma)	Blood (mg. per 100 cc.)	
				Non-protein nitrogen	Chlorides (as NaCl)
		per cent	*mg.*	*mg.*	*mg.*
16	0	44	686	26.5	460
	1	51	1238	33.0	460
	2	54	1358	116.0	390
17	0	47	489		430
	1	47	820	25.7	390
	2	49	1000	31.9	300
	3	46	681	65.0	250
18	0	50	670	46.8	450
	1	65	801	86.3	360
19	0	56	620	34.5	440
	1	60	813	41.4	350
	2	58	953	63.0	320
	3	58	905	12.2	320
	4	53	760	105.0	270
	6	55	650	126.0	220
	8	55	692	179.0	200
	10	53	725	234.0	180

obstruction of the cardia and pylorus. One animal (No. 19) showed an exceedingly low blood chloride and high non-protein nitrogen with no marked change in the fibrin.

DISCUSSION.

The very high blood fibrin level observed in this series of animals emphasizes the tissue injury occurring in such conditions. The rise

is especially striking after obstruction of the cardiac end of the stomach which we have shown is characterized by a toxemia more serious than that incident to pyloric or high intestinal obstruction.

These findings are of interest also in indicating that hepatic insufficiency is not the cause of death in high gastrointestinal tract obstruction as has been suggested (9). If there is any liver injury it must be mild since extensive liver injury without exception has been shown to give low values for fibrin.

SUMMARY.

After high gastrointestinal tract obstruction the blood fibrin increases rapidly.

The change is more marked in animals with obstruction of the cardiac end of the stomach.

The rise in fibrin parallels closely the toxemia characteristic of such conditions.

These results indicate that liver insufficiency cannot be the cause of death in such obstructions.

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THE EXCRETION OF NITROGEN AFTER UPPER GASTRO-INTESTINAL TRACT OBSTRUCTION.

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A high level of non-protein nitrogen in the blood is characteristic of the toxemia incident to upper gastrointestinal tract obstruction (1, 2). The explanation for this change is not entirely clear. Whipple, Cooke and Stearns found an increase in nitrogen excretion in the dog after intestinal obstruction (3), and no marked evidence of renal insufficiency (4). They attributed the heaping up of non-protein nitrogen in the blood to accelerated protein destruction caused by some toxic body arising in the wall of the obstructed gut. More recently there has been a tendency to doubt the existence of a toxic body and to consider the increase in the non-protein nitrogen of the blood as a true retention due entirely to renal insufficiency, possibly caused in turn by dehydration. Convincing proof for this view is lacking. The high blood urea concentration observed in children suffering from water loss produced by gastrointestinal tract disturbances, has also been interpreted as due to renal insufficiency without any adequate proof (5). Gamble and Ross (6) in studying experimentally the dehydration of rabbits, after pyloric obstruction, make a similar assumption.

The question can be settled only by determining accurately the nitrogen excretion. In experimental pyloric and intestinal obstruction this is most difficult, since collections of urine can seldom be accurately made on account of vomiting. We have shown that the toxemia incident to obstruction of the cardiac end of the stomach is similar to but more marked than that following high intestinal or pyloric obstruction (7). Since here no vomiting is possible, the urine can be quantitatively collected. We have also found that if the pylorus is obstructed for 48 hours and then released, although the vomiting

ceases, making urine collections possible, the toxemia often continues. We have determined the non-protein nitrogen excretion in six dogs with obstruction of the cardia, in two after obstruction and release of the pylorus and in two control dogs.

TABLE I.

Excretion of Nitrogen after Upper Gastrointestinal Tract Obstruction.

Dog	Duration of experiment	Blood (mg. per 100 cc.)				Urine (per kilo of body weight per 24 hrs.)		Operation
		Beginning		End		Amount	Total non-protein nitrogen	
		Urea nitrogen	Non-protein nitrogen	Urea nitrogen	Non-protein nitrogen			
	days	*mg.*	*mg.*	*mg.*	*mg.*	*cc.*	*gm.*	
1	4	....	....	....	....	13.0	0.141	None (control)
2	5	....	....	....	....	5.5	0.122	" "
				Average		9.3	0.132	
3	3	16.6	26.0	58.8	167.0	29.0	0.391	Obstruction of cardia
4	4	9.8	44.4	66.5	116.0	19.7	0.248	" " "
5	2	16.1	33.0	30.1	59.0	19.8	0.217	" " "
6	3	12.6	27.7	27.9	51.8	24.5	0.337	" " "
7	3	25.2	50.5	30.1	78.0	23.3	0.230	" " "
8	3	9.8	28.9	46.8	82.5	19.8	0.266	" " "
				Average		22.7	0.289	
9	7	38.5	59.5	11.9	35.3	41.8	0.557	Pylorus obstructed for 48 hrs. and released
10	10	98.1	132.0	14.7	25.2	25.0	0.413	" "
				Average		33.4	0.485	

The animals were kept in metabolism cages. All operations were done under anesthesia with aseptic technique. No food was given but water was allowed *ad libitum*. The non-protein nitrogen in the urine was determined by a micro Kjeldahl method (8), the urea nitrogen of the blood by the Van Slyke and Cullen modification of the Marshall method (9), and the non-protein nitrogen by the Folin and Wu method 10).

EXPERIMENTAL OBSERVATION.

The blood and urine findings are shown in Table I. All figures have been reduced to kilo of body weight per 24 hour period. The average excretion of non-protein nitrogen in the control animals per kilo for the 24 hour period is 0.132 gm. and the fluid excretion 9.3 cc. After cardiac obstruction the average of six dogs is 0.289 gm., an increase of 119 per cent. The water excretion is also increased from 9.3 cc. to 22.7 cc. per kilo per 24 hour period. The non-protein nitrogen excretion in two dogs, after obstruction and release of the pylorus, was 0.485 gm., an increase of 267 per cent, and water excretion 33.4 cc. per kilo per 24 hour period.

DISCUSSION.

In both cardiac obstruction and after obstruction and release of the pylorus there is a very marked increase in non-protein nitrogen and water excretion, as compared with the controls. With the increase in nitrogen excretion in the animals with cardiac obstruction there is also an increase in the non-protein nitrogen of the blood. These findings could only be explained by a marked increase in protein destruction. Dog 4 showed an increase in urea nitrogen from 9.8 mg. to 66.5 mg. per 100 cc. of blood. It has been shown that for every gm. of nitrogen retained as urea in a man weighing 70 kilos there is an increase of 1.33 mg. of urea per 100 cc. of blood (11). If these figures are applied to this dog the increase in urea nitrogen in the blood would in addition correspond to an excretion of 0.314 gm. of nitrogen per kilo per 24 hour period as urea alone. Mackay and Mackay (12) have shown that in fasting in rabbits there is a marked increase in protein destruction. This they consider secondary to the marked water loss since the increase is not due to kidney retention. Our animals also had a marked water loss. Whether the increased protein destruction is due to this cause or some toxic body is not determined. The nitrogen excretion, after pyloric obstruction and release of the pylorus, is high. The urea nitrogen and non-protein nitrogen of the blood was decreasing and some nitrogen was thus washed out. This could account for only a fraction of the nitrogen excreted, however.

SUMMARY AND CONCLUSION.

After obstruction of the cardiac end of the stomach and after obstruction and release of the pylorus there is a marked increase in non-protein nitrogen excretion.

This increase is due to accelerated protein destruction, which may result from the great fluid loss, or to the action of some toxic body.

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ETIOLOGY OF OROYA FEVER.

VI. Pathological Changes Observed in Animals Experimentally Infected with Bartonella bacilliformis.

The Distribution of the Parasites in the Tissues.

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Plates 11 to 14.

(Received for publication, October 20, 1926.)

Experimental infection with *Bartonella bacilliformis* in young *Macacus rhesus* monkeys varies in severity according to the susceptibility of the individual animals[1,2,3] and is of three general types: (1) severe, fatal affection characterized by extreme anemia, similar to Oroya fever in man, (2) relative benign condition, in which there is moderate anemia or none at all, accompanied by localized skin lesions resembling those of human verruga, (3) mild systemic disease, involving the lymphatic glands and blood.

The pathological changes found in human organs and tissues after death from *Bartonella bacilliformis* infection have been described by Odriozola,[4] Arce,[5] Hercelles,[6] and by Strong and his coworkers.[7] The characteristic effects are seen in the liver, spleen, lymphatic glands, and bone marrow. Hypertrophy of the liver is almost invariable,[4, 5] and the spleen is frequently enlarged,[4, 7] though it may

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be of normal size or less.[5] Infarcts are commonly found in the spleen and sometimes also in the liver.[6, 7] Strong and his coworkers note particularly the areas of degeneration in the liver, beginning about the hepatic veins, apparently due to the activity of the parasite and suggesting the presence of a toxin in the circulating blood. The lymphatic glands are enlarged everywhere[4, 5, 7] and frequently edematous. The hypertrophy is most marked in the mesenteric lymph nodes.[4] The marrow of the long bones is red or mottled with red patches,[4, 7] and distinctly softer than normal. Microscopically there is evidence of phagocytosis by endothelial leucocytes of red cells and polymorphonuclear leucocytes, also increased production of normoblasts.[7] Extensive phagocytosis by endothelial cells is found also to occur in the spleen, while the lymphatic glands often contain many large swollen endothelial cells in which the bacilliform parasites are present in large numbers.[7] Similar intracellular parasites may be found[7] in the blood vessels near ulcerated areas in the intestinal wall. The parasite has a marked predilection for the reticulo-endothelial system and sets up an active and persistent proliferation of endothelial cells in the tissues or organs which it invades.

The characteristic nodular lesions caused by *Bartonella bacilliformis* have occasionally been found to occur in the muscles and viscera as well as on the mucous membranes.[4, 5, 8, 9] Campodónico, in 1895,[10] reported the finding at autopsy, in a young child who succumbed to severe anemia during the course of verruga, of tiny red nodules in all the tissues of the body.

In the early experimental work with *Bartonella bacilliformis* only the milder types of infection were observed, and it was not until the third and fourth generations in monkeys that it was possible to induce, by massive inoculations of passage strains, a fatal disease accompanied by extreme anemia comparable with Oroya fever of man. Five animals succumbed to the infection, and, as in the case of the human disease, the conspicuous changes were found in the liver, spleen, bone marrow, and lymphatic glands. It was desirable, therefore, not only to compare the findings with those occurring in human material, but also to relate the histological changes in a particular tissue with the presence or absence of the parasite. In order better to accomplish this purpose a number of infected animals were sacrificed either at the height of disease, as evidenced by extensive local lesions and a high titer of the parasite in the blood, or during the period of convales-

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cence, when the blood findings were negative and the local lesions had subsided.

The important fact should be emphasized that tuberculosis was not encountered among the animals of the present series, notwithstanding special search was made for *B. tuberculosis* because of the possibility of confusion from this source.

Although microscopic examination of all materials—blood, organs, or tissues—was always made, the cultural method was found to be more reliable for determining the presence of *Bartonella bacilliformis*. Moreover, secondary infection is readily detected by this method. About 0.1 cc. of each of several dilutions (from 1:10 up to 1:1,000,000) of blood or saline suspension of tissue is introduced into leptospira medium, and the tubes are kept constantly at 25°C. for 4 to 5 weeks and examined periodically. Growth is often detected only after 2 to 3 weeks.

Films were always made of blood and impression smears of spleen, bone marrow, lymph glands, and liver. They were dried in air, fixed in methyl alcohol for 5 to 10 minutes, and stained with Giemsa's solution for 30 to 60 minutes.

Tissues were fixed in Regaud's fluid, and the sections stained with hematoxylin and eosin for histological study and with Giemsa's solution for detection of *Bartonella bacilliformis*.

The fourteen monkeys (*Macacus rhesus*) serving as material for the present study will be considered in three groups: (1) five animals which died of experimental infection with *Bartonella bacilliformis;* (2) five animals which showed signs of infection, locally and constitutionally, when sacrificed; (3) four animals which had shown signs of infection but were apparently in convalescence when sacrificed.

In the first group, three showed marked and two extreme anemia during the latter part of the disease or at the time of death. In Monkey 25 the red blood cells numbered 1,164,000 per c.mm. and the hemoglobin (Sahli) was 25 per cent; in Monkey 37 the red cells numbered 1,176,000 and the hemoglobin was 15 per cent. In these animals a terminal secondary bacterial infection intervened 12 and 96 hours before death.

There was moderate anemia in some of the animals of Group 2 but none in those of Group 3.

In Group 1 (fatal cases) the clinical picture was that of Oroya fever, and the pathological changes likewise proved to be similar to those

observed in the human infection. In Group 2 (non-fatal cases), the manifestations were characteristic of human verruga.

Pathological Findings in Fatal Infections with Bartonella bacilliformis.

The following outline is a composite picture of the characteristic changes noted. The findings in individual instances and the relation of the cultural to the pathological findings are recorded in Table I and in the protocols.

The duration of illness was 56 days in Monkey 17, 36 days in Monkey 25, 25 days in Monkey 30, 29 days in Monkey 35, and 41 days in Monkey 37.

External Appearances.

The skin and mucous membranes have a striking waxy yellowish appearance, and the numerous swollen lymphatic glands in the axillary and inguinal regions stand out prominently beneath the stretched skin. Pale nodules are found at the original sites of inoculation on eyebrows and abdominal skin. In an exceptional instance (Monkey 25) there were also numerous spontaneously occurring nodules on various parts of the body.

Thorax.

Lungs.—Pale yellow, normally crepitant, smooth. Congestion or edema at base in some instances. Bronchial lymph nodes greatly enlarged. Pleuræ anemic; small amount of effusion present.

Heart.—Myocardium flabby, pale, contains partially fluid pale red blood and occasionally a small clot. No petechiæ present. The pericardium contains some clear fluid. In one instance (Monkey 25) an acute pericarditis due to secondary invasion was present.

Abdomen.

Liver.—Pale, flabby in consistency, frequently somewhat enlarged, often shows yellowish mottling. Areas of necrosis and fatty degeneration are often recognizable.

Spleen.—Dark bluish red, enlarged, rather firm in consistency. Follicles indistinct. Scattered minute grayish red areas (infarctions) are present in all instances in varying numbers.

Kidneys.—Pale, with yellowish gray tint.

Adrenals.—Pale yellow.

Pancreas.—Normal in appearance.

Stomach.—Usually empty, mucosa pale.

Small Intestine.—Pale, serosa studded with numerous dark bluish swollen lymph nodes. Mucosa pale. Peyer's patches slightly hypertrophied.

Large Intestine.—Similar in appearance to small intestine, swollen lymph nodes on serosa giving it a striking aspect. No ulceration of the mucosa.

Bladder.—Normal in appearance.

Ovaries, Uterus, Testicles.—Pale, but normal.

Bone Marrow (Femur).—Dark grayish red.

Lymphatic Glands.—Entire lymphatic system hypertrophied and congested to an extraordinary degree. In the inguinal and axillary regions two or three swollen glands sometimes unite to form an irregular mass, 2 to 3 cm. in longest diameter, pink to deep red in color, and edematous. In the abdominal cavity the swollen lymph nodes are sometimes dark bluish, sometimes bluish red, and by contrast with the pale intestinal wall present a spectacular picture. They range in size from 2 to 6 mm. and are attached so firmly to the intestinal wall that they appear like verrucous nodules.

Nervous System.—The brain and spinal cord and their coverings are anemic but otherwise appear normal.

Microscopic Pathology.

Liver.—The normal pattern of cell arrangement is more or less disturbed. The hepatic cells have lost their sharp outline, stain poorly, and are vacuolated. The cells around the hepatic veins are necrotic (Fig. 1). Here and there, on the borders of the necrotic areas, between the liver cells, are engorged macrophages, which often contain erythrocytes, polymorphonuclear leucocytes, and erythroblasts. Around the portal veins are several layers of proliferated endothelial cells. In some specimens yellowish or brownish pigment in granules or small masses is found within the endothelial phagocytes or in endothelial cells lining the sinusoids. The degree of necrotic change around the hepatic veins is variable in the different animals. In some areas the liver cells are clear and homogeneous, in others coarsely granular. A few endothelial cells containing *Bartonella* were found after prolonged search in two of the five livers (those of Monkeys 17 and 30).

Spleen.—(Figs. 7, 8, 23.) Many infarctions are found, the periphery showing invasion of leucocytes. Many of the veins are occluded, owing probably to endothelial hyperplasia. The splenic nodules are reduced in size and are often scattered through the proliferating endothelial cells. Engorged macrophages containing erythrocytes are quite abundant. Yellowish brown pigment is present, some in the endothelial leucocytes, some between the splenic cells.

A number of endothelial cells containing masses of elements resembling *Bartonella bacilliformis* were found (Figs. 16, 25), but none of the swollen endothelial cells containing rods and granules such as were seen in human materials by Strong and his coworkers.

Lymphatic Glands.—The principal changes in these glands are the increase in the number of endothelial cells and the presence of numerous engorged macrophages. In the mesenteric nodes a large amount of greenish pigment is found in the phagocytic endothelial cells of the sinuses. *Bartonella bacilliformis* has been observed in a few endothelial cells in Monkeys 29 and 30 (Fig. 27).

Bone Marrow (*Femur*).—Numerous macrophages containing erythrocytes, polymorphonuclear leucocytes, and cell débris are present, and there are large numbers of normoblasts in some specimens (Monkeys 17, 25, 30). Some cells, probably endothelial, containing elements resembling *Bartonella bacilliformis* have been found in film preparations (Figs. 15, 26).

The changes are strikingly like those described as occurring in tissues from fatal cases of Oroya fever, the differences being in degree rather than in kind. For example, the changes in the liver and spleen in monkeys are decidedly less pronounced than those seen in such human materials as are at my disposal for comparison, while the reaction in the lymphatic glands is in reverse degree, the more active phagocytosis being observed in monkeys. The origin of the minute necrotic areas in the spleen may be sought in the obstruction of capillary lumina by proliferating endothelial cells.

As in the skin lesions, so also in the spleen and lymph glands, the progressive and persistent proliferation of endothelial cells is usually associated with the presence of *Bartonella bacilliformis* in considerable numbers, as detected by cultures. The extensive zonal necrosis around the veins of the liver, which is less extensively invaded by the parasite, would appear, as suggested by Strong and his coworkers, to be due to the presence of a toxin in the circulating blood.

Pathological Findings in Animals Sacrificed during the Period of Active Infection.

The five animals of Group 2 all showed signs of active infection at the time when they were killed by etherization. The period from inoculation to the time of killing was 22 days in Monkey 4, 68 days in Monkey 5, 53 days in Monkey 23, 24 days in Monkey 24, and 28 days in Monkey 29. None of the animals showed severe anemia, although in Monkey 24 there was moderate diminution both in red blood cells and hemoglobin (erythrocytes 3,432,000, hemoglobin 55 per cent). *Bartonella bacilliformis* had been cultivated from the blood of Monkey 23 in a dilution of 1:100,000 16 days after inoculation, but it could not be recovered from undiluted blood 15 days later nor at any subsequent time.

The spleen and lymphatic glands were usually affected, and also the bone marrow as far as studied; the liver was less frequently in-

volved. Except in Monkey 23, of which special mention will be made later, the other organs were always normal in appearance.

The spleen was always more or less enlarged and rather firm; in one instance the surface was rather irregular (Monkey 5). In one animal (Monkey 23) there were numerous grayish brown areas of infarction, 1 to 1.5 mm. in diameter, scattered over the organ, which was a dark bluish red (Fig. 23). The lymphatic glands were in all cases swollen and congested and approached in size those of the fatally infected animals. The bone marrow was active in three animals studied (Monkeys 5, 23, and 24). The liver was affected to a similar degree in two animals (Monkeys 23 and 24); in the other three it was apparently normal. *Bartonella bacilliformis* was seen in a few of the endothelial cells in the liver of Monkey 24. The microscopical findings in the animals of Group 2 were identical with those in the fatally infected animals except that the changes were less pronounced in some respects.

An unusually interesting observation was made in the case of Monkey 23. On the pinkish surface of the lobules of the lungs were noticed a dozen pale, grayish, semitransparent, round nodules, measuring 1 to 2 mm. in diameter, firm, and sharply demarcated from the adjacent normal lung tissue (Fig. 22). Microscopically (Fig. 11), they consisted of numerous large mononuclear cells, many of them vacuolated and filled with dark granular pigment. The capillaries in this area, which were few in number, showed endothelial thickening. Several engorged macrophages and cell débris were also present. In a few of the endothelial cells *Bartonella bacilliformis* was found (Figs. 14, 24).

Pathological Changes in Animals Sacrificed during Convalescence.

Four animals were sacrificed when apparently in the course of convalescence from a mild infection. None showed fever or local lesions, though the lymphatic glands were still swollen in all instances. The erythrocyte count ranged from 5,472,000 to 6,584,000, and the hemoglobin was 80 to 90 per cent. The animals appeared to be in excellent condition when sacrificed 58 days (Monkey 7), 49 days (Monkey 8), 40 days (Monkey 10), and 30 days (Monkey 13) after inoculation.

The changes in these animals, when still evident, were characteristic. The spleen was somewhat enlarged in three instances and in two showed definite endothelial hyperplasia; in one (Monkey 13) there was a marked increase in fibrous tissue; in another (Monkey 7) the same changes were noted as had been found in the spleens of the actively infected animals. The liver was macroscopically normal in all four animals. The lymph nodes were active in two (Monkeys 7 and 8) but quiescent in the others. The bone marrow was normal in Monkeys 8, 10, and 13, and active in Monkey 7.

The findings suggested that Monkeys 7 and 8 were still infected and that Monkeys 10 and 13 had become free from infection. Cultural studies confirmed this inference, as will appear later.

DISTRIBUTION OF BARTONELLA BACILLIFORMIS IN THE TISSUES IN THE EXPERIMENTAL INFECTION.

In Oroya fever *Bartonella bacilliformis* persists in the blood until the patient's death. Whether or not various organs and tissues also contain the living parasite has not been determined. The pronounced changes found in certain organs have been assumed to be due either to invasion by the parasite or to the action of a toxin circulating in the blood. Cultural determination of the distribution of the organism in the tissues of experimental animals was, therefore, of special usefulness in establishing a relation between the pathological processes and the presence of the parasite. The results of cultural studies and of examination for pathological changes are recorded in parallel columns in Table I.

As the analysis shows, *Bartonella bacilliformis* was recovered from blood, liver, spleen, lymphatic glands, bone marrow, and local lesions in all instances of fatal infection, except when cultures were lost through contamination. The parasite was not so generally distributed in animals sacrificed during the course of an apparently nonfatal infection, though the local lesions and the changes in the spleen were rather severe. In Monkey 23 the parasite had disappeared from the blood, owing probably to the development of a blood immunity, but persisted in the lymphatic glands and spleen, while in Monkey 29 the blood and nodular tissue contained it but the organs were all free

TABLE I.

	M. rhesus No.	Blood		Liver		Spleen		Lymph gland		Bone marrow		Nodule	
		Anemia	Culture	Degree of histological change	Culture	Degree of histological change	Culture	Degree of histological change	Culture	Degree of histological change	Culture		Culture
Group 1 (fatally infected)	17	+++	+	+++	Cont.	+++	+	+++	+	+++	+	Present	+
	25	++++	Cont.	++++	"	+++	Cont.	+++	Cont.	++++	Cont.	"	+
	30	++	+	+++	+	+++	+	+++	+	+	+	"	+
	35	+	+	++	+	+	+	+	+	+++	+	"	+
	37	++++	Cont.	+++	Cont.	+++	Cont.	+++	Cont.	+++	Cont.	"	+
Group 2 (sacrificed during active stage of infection)	4	...	+	−	...	+	...	++	...	...	...	"	+
	5	+	+	−	−	++	+	++	+	++	+	"	+
	23	−	−	++	Cont.	++++	+	+++	+	++	+	"	+
	24	++	+	++	+	++	+	+	+	++	+	"	+
	29	+	+	−	−	−?	−	<+	+	...	...	"	+
Group 3 (sacrificed during convalescence)	7	−	+	−	Cont.	<+	+	<+	+	...	+	Had disappeared	
	8	−	−	−	−	−	−	<+	+	−	−	"	"
	10	−	−	−	−	−	−	−	−	−	−	"	"
	13	−	−	−	−	−	−	−	−	−	−	"	"

Anemia + ++ + = r.b.c. 1,000,000 to 1,500,000, Hb. 15 to 25 per cent; +++ = r.b.c. less than 3,000,000; ++ = less than 4,000,000; + = less than 5,000,000; − = over 5,500,000.

Degree of change in tissue varies from marked (++++) to slight (+).

Cont. = secondary bacterial contamination.

Summary of Pathological

M. rhesus No.		Liver
17	Inoculation Mar. 15, 1926. Died after 57 days. Fever still present. Nodules active. Anemia marked (r.b.c. 3,112,000, Hb. 50 per cent). Blood culture + at autopsy. Nodule culture + at autopsy	Pale, somewhat enlarged. Structure dis turbed, cells along central zones poorl stained. Perivascular proliferation mod erate. Greenish pigment present. *B. bacilliformis* found in a few endothelia cells. Culture contaminated
25	Inoculation Mar. 23, 1926. Died after 36 days. Course practically afebrile. Local lesions extensive, partly generalized. Anemia very severe (r.b.c. 1,624,000, Hb. 25 per cent). Blood (1:100,000) culture + 12 hrs. before death. Culture of nodules taken at autopsy +	Pale, soft, mottled, perhaps enlarged Structure fairly well preserved but pro nounced central necrosis throughout Numerous phagocytes, polymorphon clears, and pigments present. Hepat cells vacuolated. Culture contaminate
30	Inoculation Mar. 31, 1926. Died after 25 days. Fever present. Nodules small but active. Blood (1:10,000 dilution) culture + 3 days before death. Anemia moderate (r.b.c. 3,920,000, Hb. 60 per cent). Blood and nodules taken at autopsy yielded cultures	Pale, appears fatty. Normal structure lo cells stain poorly, vacuolated and d sociated. Endothelial hyperplasia; so cells contain *B. bacilliformis*. Ma phagocytes in sinusoids. Culture +
35	Inoculation Apr. 12, 1926. Died after 29 days. Fever still present. Nodules small but active. Anemia slight (r.b.c. 4,776,000, Hb. 80 per cent). Blood (1:100) culture + 10 days before death. Blood and nodules taken at autopsy yielded cultures	Probably paler than normal. General str ture disturbed. Marked dissociation vacuolation of cells. Invasion of si soids by phagocytes. Proliferation endothelial cells. Culture +

and Cultural Findings.

Spleen	Lymphatic glands	Bone marrow	Remarks
Enlarged, rather firm. Follicles reduced. Endothelial hyperplasia. Active phagocytosis. Fibrin masses and pigments present. *B. bacilliformis* found. Culture positive	All lymph nodes much swollen and congested. Endothelial hyperplasia and active phagocytosis throughout. Culture +	Dark grayish red. Very active phagocytosis. Numerous normoblasts and polymorphonuclear leucocytes present. Culture +	*B. bacilliformis* found in all tissues studied
Slightly enlarged, soft. Follicles smaller, outlines of many lost. Numerous perifollicular necrotic foci. Marked endothelial hyperplasia. Active phagocytosis of degenerated polymorphonuclears and erythrocytes. Many normoblasts present. Culture contaminated	Marked swelling. throughout. Mesenteric nodes appear like scattered or clustered peas but bluish in color. Pronounced proliferation of endothelial cells and active phagocytosis. Culture contaminated	Dark grayish red. Active phagocytosis. Polymorphonuclears and normoblasts present. Culture contaminated	Terminal bacterial infection (pericarditis) intervened after heart puncture 24 hrs. before death
Enlarged. Follicles thinned and diffuse in places. Endothelial elements increased around vessels; small foci with fibrinous exudate and cell débris. Culture +	General swelling. Endothelial hyperplasia, active phagocytosis, greenish pigments. *B. bacilliformis* found in a few endothelial cells. Culture +	Grayish red. Active phagocytosis. Polymorphonuclears and normoblasts present. Culture +	*B. bacilliformis* found in all tissues studied
Dark and soft. Follicles ill defined, pervaded by increased endothelial cells. Active phagocytosis. Culture +	Swelling and congestion throughout. Endothelial hyperplasia. Phagocytosis. Culture +	Grayish red. Active phagocytosis. Culture +	*B. bacilliformis* found in all tissues studied

Summary of Pathological

M. rhesus No.		Liver
37	Inoculation Apr. 17, 1926. Died after 41 days. Fever still present. Small nodule on leg. Blood (1:1,000) + 96 hrs. before death; r.b.c. 1,664,000, Hb. 25 per cent. Autopsy blood contaminated; anemia extreme (r.b.c. 1,176,000, Hb. 15 per cent). Nodules (autopsy) +	Pale and soft. General structure much disturbed. Cells stain very poorly, necrotic along central zones. Small masses of polymorphonuclear leucocytes scattered in sinusoids, where many loaded phagocytes are present. Endothelial proliferation marked. Culture contaminated
4	Inoculation Dec. 8, 1925. Killed after 22 days. Fever present. Nodules active. Blood culture at autopsy +. Nodules at autopsy +	Apparently normal. No culture made
5	Inoculation Dec. 11, 1925. Killed after 68 days. Had become afebrile. Nodules large and active. R.b.c. 4,856,000, Hb. 85 per cent. Blood culture and nodules (autopsy) +	Apparently normal. No culture made
23	Inoculation Mar. 9, 1926. Killed after 53 days. Fever present. Nodules active, extensive. Blood + in 1:100,000, 40 days previously, − 22 days previously and at autopsy. Nodules (autopsy) +	General structure irregular; some ill stained foci. Numerous polymorphonuclears. Moderate endothelial hyperplasia. Culture contaminated
24	Inoculation Mar. 19, 1926. Killed after 24 days. Fever present. Nodules active. Anemia moderate (r.b.c. 3,432,000, Hb. 55 per cent). Blood (1:10) and nodules at autopsy +	Pale, mottled. Moderate degree of central necrosis, uniform structure lost in places. Endothelial proliferation around vessels. *B. bacilliformis* found in a few endothelial cells. Culture +

nd Cultural Findings.

Spleen	Lymphatic glands	Bone marrow	Remarks
rk and firm. Only a few follicles preserved. Necrotic areas (infarctions) showing active phagocytosis found. Increase in endothelial cells. Culture contaminated	Swollen and congested. Endothelial hyperplasia. Active phagocytosis. Polymorphonuclear leucocytes present. Culture contaminated	Grayish red. Active phagocytosis and increase in polymorphonuclear leucocytes. Culture contaminated	Terminal secondary infection by strain of *B. paratyphosus* A 48 hrs. before death
mewhat swollen and firm. Considerable increase in endothelial elements. No culture made	Enlarged and congested everywhere. Definite endothelial hyperplasia; active phagocytosis; pigments. No culture made	Not examined	Pure infection with *B. bacilliformis*
larged, granular surface. Follicles ill defined in many places. A few necrotic areas. Active phagocytosis. Culture +	Moderately swollen and congested. Phagocytosis active. Hyperplasia of endothelial cells. Culture +	Grayish red. Active phagocytosis. Normoblasts present. Culture +	*B. bacilliformis* found in all tissues studied except liver. Animal moderately resistant
mewhat enlarged, shows numerous brown patches of infarction. Many perifollicular necrotic areas. Active phagocytosis. Pigments present. Endothelial hyperplasia. Culture +	Greatly swollen and congested. Hyperplasia of endothelial elements. Very active phagocytosis. Culture +	Grayish red. Phagocytosis active. Many polymorphonuclear leucocytes. Culture +	Note absence of *B. bacilliformis* in blood and persistence in other tissues
larged, bluish red. Many follicles diffuse, interspaced by proliferated endothelial cells. Active phagocytosis. Many normoblasts. Culture +	Swollen and congested. Endothelial hyperplasia and phagocytosis. Culture +	Grayish red. Active phagocytosis. Numerous polymorphonuclears and normoblasts present. Culture +	*B. bacilliformis* found in all tissues studied. Animal quite susceptible

Summary of Pathological

M. rhesus No.		Liver
29	Inoculation Mar. 30, 1926. Killed after 28 days. Fever had been present for 2 wks. Nodules very active. Blood (1:1,000) + day before autopsy. Anemia noticeable. R.b.c. 4,704,000, Hb. 70 per cent. Blood and nodules at autopsy +	Mottled. Microscopically appears normal. Culture −
7	Inoculation Dec. 21, 1925. Killed after 58 days. Fever had subsided 10 days previously. Nodule removed 30 days previously +. Blood + 8 days previously. Blood taken at autopsy +. R.b.c. 5,472,000, Hb. 80 per cent	Appears normal. Culture contaminated
8	Inoculation Dec. 30, 1925. Killed after 49 days. Fever had subsided 7 days previously. Nodules absent. Blood + 14 days previously, − at autopsy. R.b.c. 5,496,000, Hb. 80 per cent	Appears normal. Culture −
10	Inoculation Jan. 8, 1926. Killed after 40 days. Fever had subsided 14 days previously, nodules 3 wks. previously. Blood (1:100) + 30 days previously, − at autopsy. R.b.c. 6,584,000, Hb. 65 per cent	Appears normal. Culture −
13	Inoculation Jan. 19, 1926. Killed after 30 days. Fever had subsided 10 days previously. No nodules. Blood + 19 days previously, − 8 days previously, − at autopsy. R.b.c. 6,104,000, Hb. 90 per cent	Appears normal. Perivascular infiltrati present. Culture −

from it. In the latter instance the organism had apparently not yet invaded the organs.

Of the four animals in which fever and local lesions had been present but had subsided, two (Monkeys 7 and 8) proved to be still carrying *Bartonella bacilliformis* in one tissue or another. Monkey 8 is especially interesting, as the latent infection was demonstrable only in the lymphatic glands. The other two animals (Monkeys 10 and 13) had

and Cultural Findings.

Spleen	Lymphatic glands	Bone marrow	Remarks
Enlarged, bluish red. Follicles appear to be of normal size and structure. Moderate endothelial hyperplasia. Numerous normoblasts. Culture —	Swollen and congested. Hyperplasia of endothelial elements, some phagocytosis. Culture +	Not examined	Liver and spleen had probably not yet been invaded. Animal rather resistant
Enlarged, follicles prominent. Only slight hyperplasia of endothelial cells microscopically. Follicles apparently normal. Culture +	Moderate swelling. Slight endothelial proliferation. Culture +	Grayish red. No sections. Culture +	*B. bacilliformis* found in most tissues studied: evidence of chronicity of infection
Appears normal. Culture —	Marked swelling and congestion. Hyperplasia of endothelial cells. Phagocytosis active. Culture +	Grayish yellow. Apparently normal. Culture —	*B. bacilliformis* found only in lymph nodes. Animal apparently convalescing
Probably enlarged. Microscopically normal. Culture —	Definitely enlarged, but pale. General hyperplasia. Culture —	Grayish yellow. Normal. Culture —	Had recovered from infection mildness of which was probably due to resistance of animal
Slightly enlarged (?); surface uneven, firm. Definite increase in fibrous tissues. Hyperplasia of endothelial cells. Culture —	Considerable swelling and congestion. Hyperplasia. Culture —	Grayish yellow. Apparently normal. Culture —	Recovered from mild infection. High resistance of animal noted

completely recovered, as indicated by the histological findings and proven by the negative results of cultural experiments.

Brief protocols of all the animals of the present series are recorded above.

SUMMARY.

The pathological changes observed in the organs in *Macacus rhesus* monkeys which have succumbed to severe infection with *Bartonella*

bacilliformis are similar to those found in human organs in persons dying of Oroya fever.

The characteristic changes in the liver are the zonal necrosis of the cells around the hepatic veins, involving active macrophagocytosis of invading polymorphonuclear leucocytes in the necrotic areas, and a marked endothelial hyperplasia in the sinusoids or around the portal veins. In some instances there is fatty infiltration of hepatic cells.

In the spleen persistent hyperplasia of the endothelial cells of the capillaries leads to the formation of minute foci of infarction, owing to occlusion of the lumina. The follicles are dispersed or reduced, and there is an active macrophagocytosis of cellular débris, polymorphonuclear leucocytes, and erythrocytes. In some specimens an increase in normoblasts is noted. Pigment is sometimes present.

The lymphatic system shows general progressive endothelial hyperplasia, with active invasion of macrophages which contain polymorphonuclear leucocytes, erythrocytes, and greenish or dark pigments.

In the bone marrow there is increased activity of macrophagocytes. Numerous normoblasts are found in some instances.

In one monkey, sacrificed during the course of injection, small, verruga-like nodules were found in the lungs and spleen.

Bartonella bacilliformis has been detected microscopically, though in small numbers, in all tissues showing histological changes. Parallel cultural determinations of the presence of *Bartonella bacilliformis* in the blood, liver, spleen, lymphatic glands, bone marrow, and local lesions established the relationship between the pathological conditions and the presence of the parasite. The organism seems to persist longest in the lymphatic glands. Cultural methods offer a simple and conclusive means for the determination of the presence or absence of the infecting organism.

EXPLANATION OF PLATES.

PLATE 11.

FIG. 1. Experimental Oroya fever. Section of liver from *M. rhesus* 25, showing a rather characteristic zonal necrosis of the cells around the hepatic veins. Giemsa's stain. × 50.

FIG. 2. The same section, × 182.

FIG. 3. Human Oroya fever. Section of liver from the case (S. A. 15) from

which the strain of *Bartonella bacilliformis* employed in these investigations was isolated. The necrotic changes of the liver cells are more diffuse than in the experimental infection, but there is a definite tendency to a central type. Giemsa's stain. × 50.

FIG. 4. The same section, × 182.

FIG. 5. Human Oroya fever. Section of liver from the collection of the Harvard School of Tropical Medicine. Courtesy of Professor R. P. Strong. This patient was free from any verruga lesions and was regarded by the Harvard Commission as a pure case of Oroya fever. The section shows the characteristic central necrosis. Note the striking resemblance between this preparation and that of the monkey liver in Fig. 1. × 50.

FIG. 6. The same section, × 182.

PLATE 12.

FIG. 7. Experimental Oroya fever. Section of spleen from *M. rhesus* 23. A splenic follicle in an infarction zone, showing a perifollicular necrosis and the invasion of the follicle by proliferating endothelial cells, endothelial phagocytes, and polymorphonuclear leucocytes. Giemsa's stain. × 182.

FIG. 8. Experimental Oroya fever. Section of spleen from *M. rhesus* 25. An area of infarction where the splenic nodule has practically disappeared. Giemsa's stain. × 182.

FIG. 9. Human Oroya fever. Section of spleen, for comparison. A splenic follicle within the infarction, showing a general disorganization, necrosis, and phagocytic invasion. Giemsa's stain. × 182.

FIG. 10. Human Oroya fever. Section of spleen from another case. The structure of spleen is greatly disturbed, shows general endothelial hyperplasia, and the presence of macrophagocytes and polymorphonuclear leucocytes. Note the striking resemblance between these lesions and those shown in Figs. 7 and 8. Giemsa's stain. × 182.

FIG. 11. Section of one of the nodules in the lung of *M. rhesus* 23. Giemsa's stain. × 50.

FIG. 12. The same section, × 182, showing the proliferation of endothelial cells within the nodule.

PLATE 13.

FIG. 13. *Bartonella bacilliformis*, colonies from a blood agar slant grown for 6 days 25°C. Smear preparation stained with Giemsa's solution for 1 hour, showing the pleomorphism of the organism. Inserted here for comparison with the intracellular forms. × 1,000.

FIG. 14. Film preparation from a nodule in the lung of *M. rhesus* 23, showing irregular masses of *Bartonella bacilliformis* within the cytoplasm of an endothelial cell. Giemsa's stain. × 1,000.

FIG. 15. Film preparation from the bone marrow (femur) of *M. rhesus* 23,

showing *Bartonella bacilliformis* within one of the endothelial cells. Giemsa's stain. × 1,000.

FIG. 16. Film preparation from the spleen of *M. rhesus* 23, showing a cell (endothelial?) containing several small masses (*Bartonella bacilliformis?*). Giemsa's stain. × 1,000.

FIG. 17. Section of an experimental skin nodule produced in *M. rhesus* with a strain of *Bartonella bacilliformis* from verruga,[11] showing a dense mass of the parasites within an endothelial cell. Giemsa's stain. × 1,000.

FIG. 18. Section of an experimental subcutaneous lesion produced in the chimpanzee[3] with the strain of *Bartonella bacilliformis* from Oroya fever, showing irregularly scattered masses of *Bartonella bacilliformis*. Giemsa's stain. ×1,000.

FIG. 19. Section of a skin lesion experimentally produced in an ourang-utan,[3] showing a small mass of *Bartonella bacilliformis* (below) and a dense mass of granules, probably of a mast cell (above). Giemsa's stain. × 1,000.

FIG. 20. Human Oroya fever. Section of lymphatic gland, showing two swollen endothelial cells, loaded with minute granules, projecting into the lumen of a capillary vessel. Giemsa's stain. × 1,000. From Case S. A. 15.

FIG. 21. Human Oroya fever. Section of lymphatic gland, showing the swollen endothelial cells lining a capillary vessel in an oblique section. These cells contain the very minute granules first described by the Harvard Commission as an intracellular phase in the life cycle of *Bartonella bacilliformis*. (Courtesy of Professor Strong.) Giemsa's stain. × 1,000.

PLATE 14.

FIG. 22. The lungs of *M. rhesus* 23, showing the pale grayish nodules on both lungs. They measured 2 to 3 mm. in diameter. In the middle, near the trachea, is a grayish lymph node of the size of a pea. Natural size.

FIG. 23. The spleen of the same monkey, showing numerous infarctions. Natural size.

FIG. 24. *Bartonella bacilliformis* in an endothelial cell in a film preparation from one of the nodules in the lung of *M. rhesus* 23. Giemsa's stain. × 1,750.

FIG. 25. *Bartonella bacilliformis* in an endothelial cell. Film preparation from the spleen of *M. rhesus* 23. Giemsa's stain. × 1,750.

FIG. 26. *Bartonella bacilliformis* (*?*) in an endothelial cell of the bone marrow (femur) of *M. rhesus* 24. Film preparation. Giemsa's stain. × 1,750.

FIG. 27. *Bartonella bacilliformis* in an endothelial cell of the lymph gland of *M. rhesus* 29. Section. Giemsa's stain. × 1,750.

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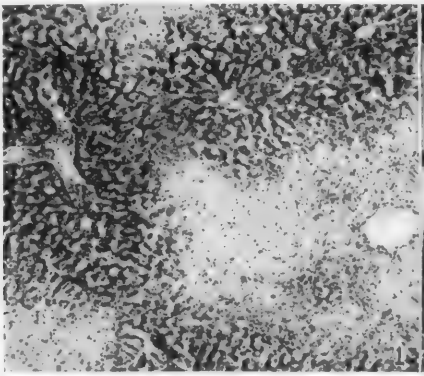
[11] Noguchi, H., *J. Exp. Med.*, 1927, xlv, 175.



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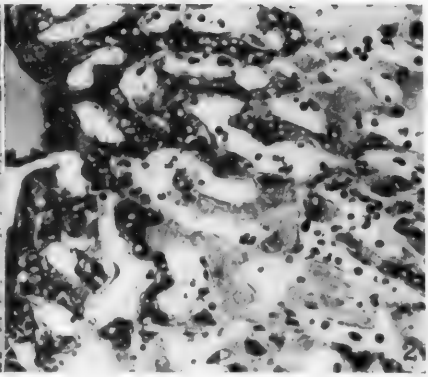

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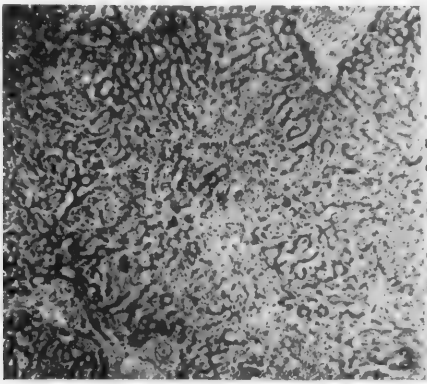

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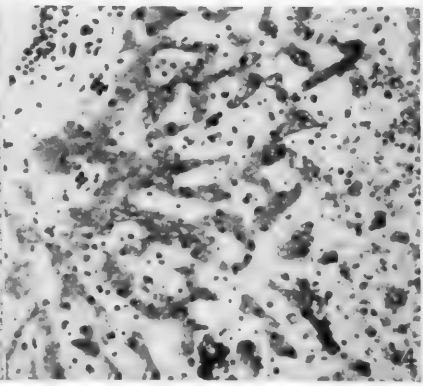
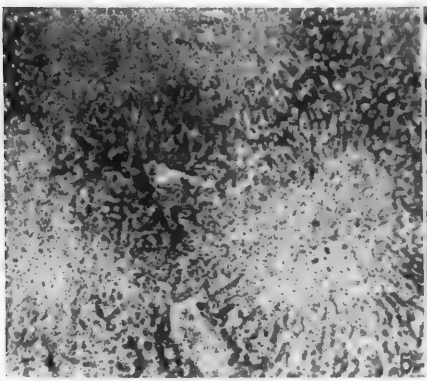
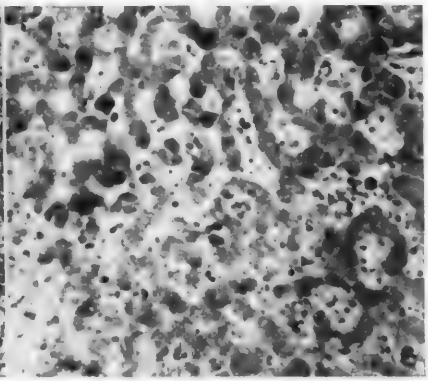


Liver, human Oroya fever. × 182.

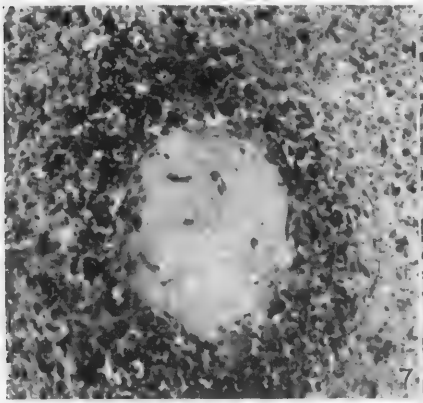


Liver, human Oroya fever. × 50.

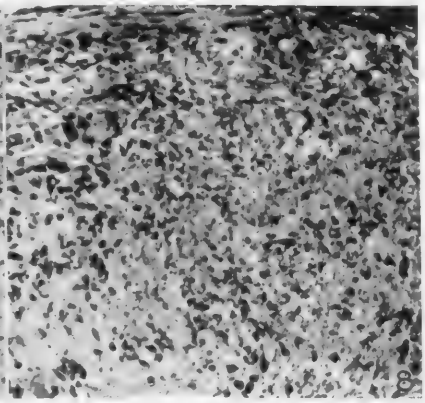


Liver, human Oroya fever. × 182.

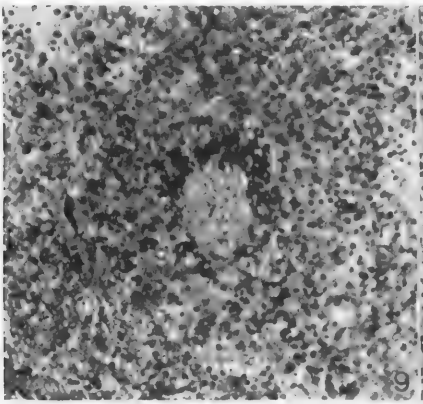
(Noguchi: Etiology of Oroya fever. VI.)



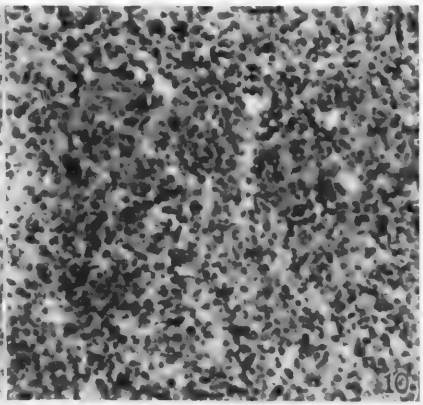
Spleen, *M. rhesus* 23. × 182.



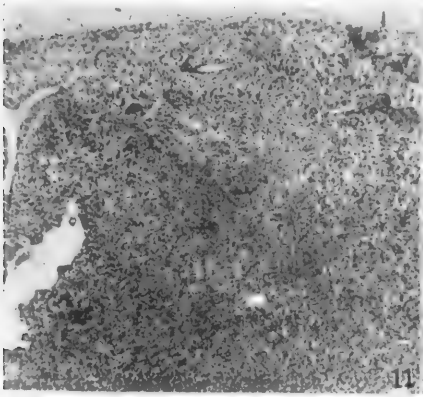
Spleen, *M. rhesus* 25. × 182.



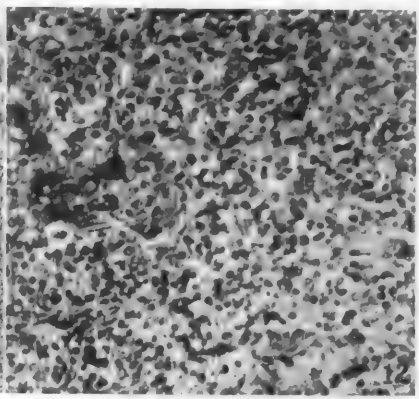
Spleen, human Oroya fever.



Spleen, human Oroya fever.



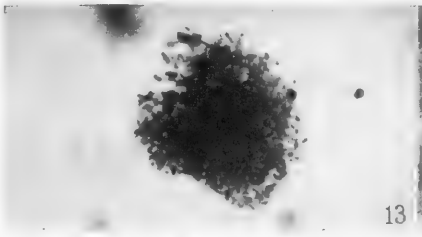
Lung nodule, *M. rhesus* 23. × 50.



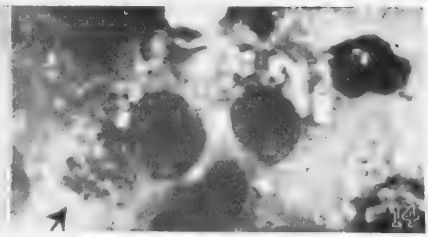
Lung nodule, *M. rhesus* 23. × 182.

(Noguchi: Etiology of Oroya fever. VI.)

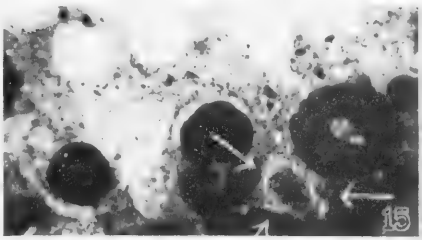
Bartonella bacilliformis. Giemsa's stain. × 1,000.



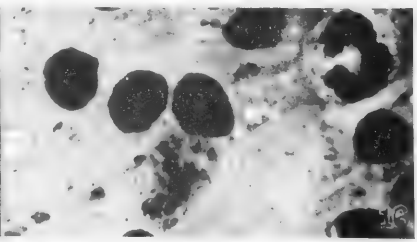
In culture, for comparison.



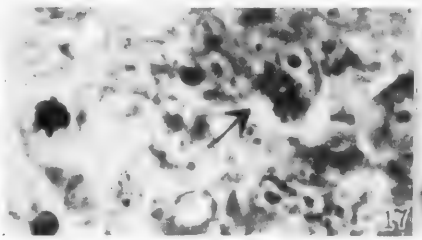
In lung nodule, *M. rhesus* 23.



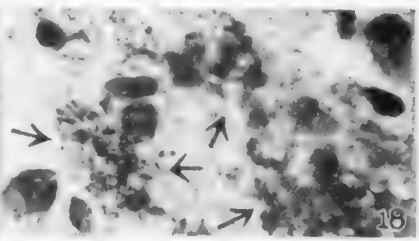
In bone marrow, *M. rhesus* 23.



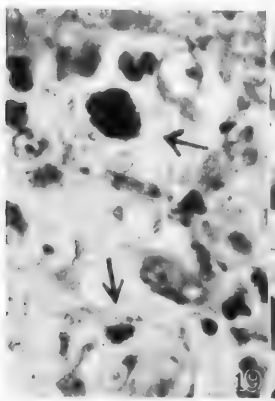
In spleen, *M. rhesus* 23.



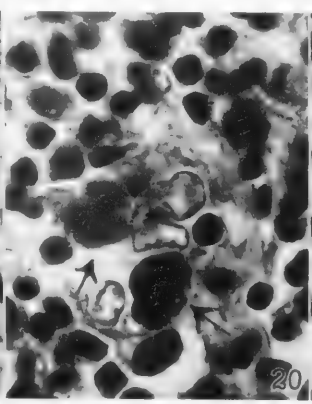
In skin nodule, *M. rhesus*.



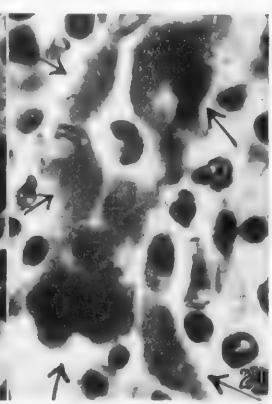
In skin nodule, chimpanzee.



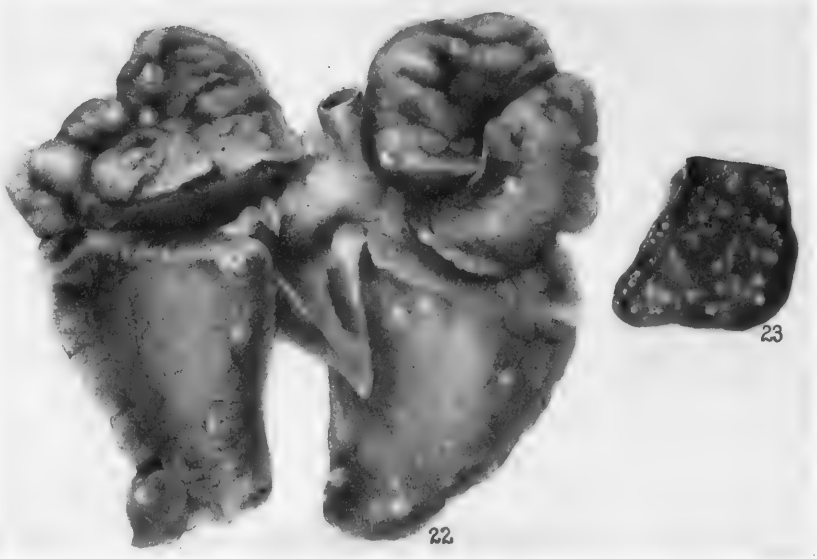
In skin lesion, ourang-utan.



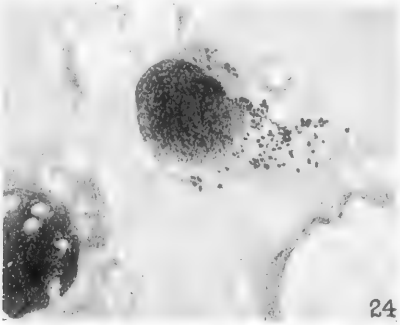
In human lymphatic gland.



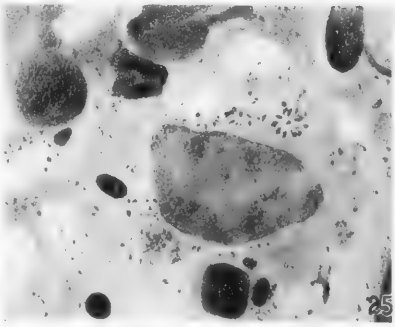
In human lymphatic gland.



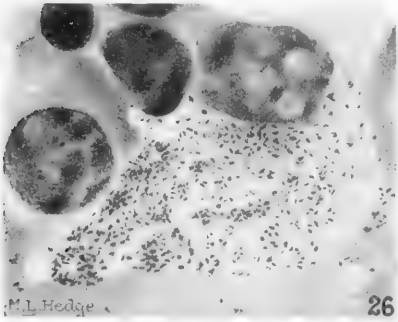
Lesions in lungs and spleen, *M. rhesus* 23.



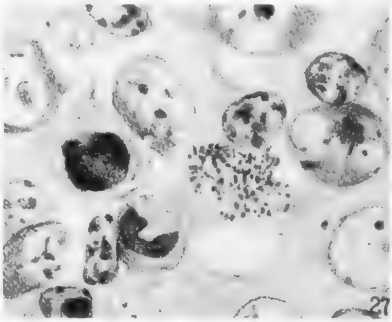
Bartonella, lung nodule, No. 23.



Bartonella, spleen, No. 23.



Bartonella (?), bone marrow, No. 24.



Bartonella, lymph gland, No. 29.

ETIOLOGY OF OROYA FEVER.

VII. The Response of the Skin of Macacus rhesus and Anthropoid Apes to Inoculation with Bartonella bacilliformis.

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In animals experimentally infected with *Bartonella bacilliformis*,[1-4] spontaneous involvement of the skin is far less frequent, and rarely so extensive, as in human verruga. In man the organism probably enters the body through the skin, and, after a preliminary multiplication elsewhere—perhaps in the lymphatic glands—invades the skin from within, giving rise to a persistent endothelial hyperplasia. In animals, when *Bartonella bacilliformis* is introduced locally, a lesion arises which may in time assume dimensions as great as the human verruga nodule. But notwithstanding the persistence of the lesion for many months, and the fact that the surrounding skin is frequently bathed in blood resulting from mechanical injury to the lesion, no new nodules develop. Hence it becomes of interest to determine whether the resistance of the skin in monkeys is due to an anergic condition, such as exists in syphilis, or to inability of the parasite to invade the intact skin.

Two other questions presented themselves in this connection, (1) whether it would be possible to induce verruga formation by injuring an area of normal skin in an animal having active skin nodules elsewhere and carrying *Bartonella bacilliformis* in the blood, and (2) whether autoinoculation would be successful in actively infected

[1] Noguchi, H., and Battistini, T. S., *J. Exp. Med.*, 1926, xliii, 851.
[2] Noguchi, H., *J. Exp. Med.*, 1926, xliv, 697.
[3] Noguchi, H., *J. Exp. Med.*, 1926, xliv, 715.
[4] Noguchi, H., *J. Exp. Med.*, 1926, xliv, 729.

animals, *i.e.*, whether skin immunity develops during the course of infection, and if so, at what time.

Resistance of the Intact Skin to Bartonella bacilliformis.

The question whether *Bartonella bacilliformis* is capable of inducing lesions when applied to the surface of the intact skin is one of practical importance. To test the point, we applied to intact areas of skin whatever material was being inoculated elsewhere. The infectious material was rubbed on two separate areas, each several cm. square, and usually near the sites of inoculation by scarification or intradermal injection. No cutaneous lesions were ever induced by this method, though lesions invariably developed at the sites of the intradermal injections and in many instances also on the areas inoculated by scarification.

Although unusually extensive lesions (*verruga mular*) are often produced by application of infectious material to scarified areas of the skin, there have been many instances in which this mode of inoculation failed, while typical lesions (*verruga nodular*) always arose at the sites of intradermal injection. Hence it is desirable always to use both methods of inoculation.

Chimpanzee,[3] *Pan leucoprymnus*, about 4 years old. *Jan. 19, 1926*, abdominal skin on the right side was shaved and two areas, each about 3 × 4 cm. square, were scarified and smeared with a saline suspension of the skin nodule* of *M. rhesus* 3. At the same time two adjacent unscarified areas were rubbed with the same suspension. *Mar. 1, 1926*, pinkish, raised indurated lesions had become noticeable on both scarified areas. *Bartonella bacilliformis* was demonstrated in the lesions, both in sections and by culture. No lesions developed on the areas which were not scarified before being smeared with the suspension.

Ourang-utan,[3] *Pongo pygæmus*, about 3 years old. *Apr. 17, 1926*, abdominal skin shaved and an area (2 × 3 cm.) on the right lower quadrant scarified and rubbed with a piece of nodule from the eyebrow of *M. rhesus* 23. An unscarified area on the upper quadrant was vigorously rubbed with the same material. *May 20, 1926*, pinkish, raised, linear lesions, rather pale, have been present on the scarified area for some time. *Bartonella bacilliformis* was demonstrated in the lesions both by culture and in sections. No lesions developed on the unscarified smeared area.

M. rhesus 18.[2] *Feb. 15, 1926*, right eyebrow and skin of right abdomen shaved and scarified and a saline suspension of the nodule of *M. rhesus* 5 applied. Adja-

* Nodules were removed under ether anesthesia.

cent unscarified areas were rubbed with the same material. Extensive nodules of deep red color developed on the scarified area of the eyebrow within a few weeks and numerous punctiform red miliary nodules on the scarified abdominal skin. The abdominal lesions disappeared within a month, the nodules on the eyebrow remained until the middle of June, when they became small, pale, and fibrous. *Bartonella bacilliformis* was demonstrated in the lesions both by culture and in sections. No lesions developed on the unscarified smeared areas.

M. rhesus 23.[5] *Mar. 9, 1926*, left eyebrow shaved and a scarified area smeared with a piece of the nodule from the eyebrow of *M. rhesus* 18. An adjacent unscarified area was vigorously rubbed with the same piece of tissue. Within 2 to 3 weeks large protruding lesions had appeared on the scarified area, and these progressed to considerable size during the following weeks. *Bartonella bacilliformis* was found in the sections of the lesion. No lesion appeared outside of the scarified area.

Relation of Injuries to Localization of Lesions.

Whether or not mechanical factors—friction, exposure to minor injury—are involved in the spread of the skin lesions in human verruga is not known. In animals these devices appear to have no influence, as shown by repeated failure to induce localized lesions in infected animals by scarifying the skin or introducing foreign substances (agar, culture medium) intradermally during the height of the infection, when *Bartonella bacilliformis* was demonstrable in the blood. In one unusually susceptible animal, *M. rhesus* 25,[2] in which spontaneous miliary nodules developed, scarification of the abdominal skin had no influence on the course of the general eruption. Similar attempts to induce nodule formation failed also in the chimpanzee, the ourang-utan, and in *M. rhesus* 18,[2] which had at the sites of scarification on the eyebrow one of the most extensive local lesions (*verruga mular*) observed in the course of the experimental work with *Bartonella bacilliformis*.

It is evident that in experimental animals *Bartonella bacilliformis* cannot be made to localize in an area of skin injured either by scarification or by intradermal inoculation of foreign substances, though present in the circulating blood and in the lymph channels, as well as in the skin lesions. Injuries of the skin are not equivalent to the deposition of concentrated infectious material on the scarified skin or in the cutaneous tissues.

[5] Noguchi, H., *J. Exp. Med.*, 1927, xlv, 437.

Autoinoculability and Superinfection.

In a disease like verruga peruana, which persists over a long period, there must be a steady process of self-infection. The manner in which the cutaneous lesions spread has not been definitely determined, but it is certain that the skin must retain its susceptibility during periods of remission and relapse. The skin of *Macacus rhesus* and that of the apes is relatively so insusceptible to infection with *Bartonella bacilliformis* that spontaneous eruptions (*verruga miliar*) seldom occur, but it has been possible to induce well marked nodular lesions (*verruga nodular*) in actively infected animals by intradermal inoculation of suspensions of nodular tissue either from the same or from another animal. Such nodules never attained the size of the primary lesions, and they usually began to recede earlier. In no instance was it possible to induce any lesions on scarified areas during the course of infection, though the materials employed induced typical lesions in control animals.

In infections with the strain of *Bartonella bacilliformis* derived from Oroya fever, the existence of active lesions considerably reduced the susceptibility of the skin to subsequent inoculation. Moreover, once the animals were free from *Bartonella bacilliformis*, they were completely refractory to reinoculation, hence the reduction in susceptibility during the course of illness is due to a partial immunity, not to a state of anergy.

On the other hand, in infections with the strain of *Bartonella bacilliformis* from a case of verruga,[6] there have been exceptional instances in which a preexisting infection did not prevent the development of the most severe type of local infection after reinoculation with the same (verruga) strain. *M. rhesus* 33 and *M. rhesus* 34 showed this type of reaction.

M. rhesus 33 was inoculated on Apr. 7, 1926, with the saline suspension of a human verruga nodule (Case P 5). Blood taken on Apr. 16 and again on May 27 yielded (in 1:10 dilution) cultures of *Bartonella bacilliformis*.

On June 3 the animal was inoculated intradermally and by scarification with the suspension of a nodule from the eyebrow of *M. rhesus* 41 (second generation of the same strain). On June 24 the lesions had already developed at the sites of inoculation, and by July 1 they were very large and active.

[6] Noguchi, H., *J. Exp. Med.*, 1927, xlv, 175.

M. rhesus 34 was inoculated on Apr. 10, 1926, with the saline suspension of the nodule of Case P 5. Cultures was obtained from diluted (1:10) blood on April 28, May 12, and May 27. By June 29 a large nodule had developed on the tail.

On June 3 the animal was inoculated intradermally and by scarification with the suspension of the eyebrow nodule of *M. rhesus* 41. *Bartonella bacilliformis* was cultivated from a 1:100 dilution of blood taken on June 20. Large lesions had appeared at the sites of inoculation at this time, and by July 1 the lesions had become unusually extensive.

As these instances show, it is not possible to make any general statement with regard to the partial immunity observed in most animals when passing through a protracted course of infection with *Bartonella bacilliformis*. Immunity may develop so slowly in some animals as not to interfere with the course of a superinfection.

Pathogenesis of the Skin Lesions.

The verruga-like skin lesion produced by *Bartonella bacilliformis* in anthropoid apes and *M. rhesus* is a slowly progressing infectious angioendothelioma and owes its origin to a delicate reciprocal relation between the endothelial cell and the microorganism. *Bartonella bacilliformis* finds within the cytoplasm of endothelial cells most favorable conditions for its multiplicative phase, and once lodged there multiplies steadily, though perhaps rather slowly, eliciting certain reactions on the part of the infected cells. Since these parasitized cells remain alive for a long time and undergo active mitosis, it is clear that the parasite furnishes a mild type of stimulus which leads to continuous proliferation of the cells. The lesion thus started continues to grow until local as well as general immunity intervenes to terminate the peculiar association of cells and parasite.

The evolution of the verruga lesion in experimental animals may be divided into (1) the incubation period, (2) the initial stage, (3) the mature vascular stage, and (4) the regression. These divisions are of course arbitrary, one stage passing into the next imperceptibly, yet each at its height has its special feature.

Incubation Period.—No instance has been observed in which the lesion was macroscopically recognizable within less than 10 days after inoculation. Usually a trace of induration can be detected after 10 to 14 days, though in some cases the incubation period is as long as 3 weeks.

Initial Stage.—When the nodular lesion, which becomes noticeable as a slightly raised area, 1 to 2 mm. in diameter, has reached a size of 4 to 6 mm. and protrudes 2 to 3 mm. beyond the surrounding normal skin, the color becomes slightly pinkish, and edema of the surrounding tissues becomes evident. The nodule is now firm and well defined to the touch and can be easily separated from the overlying skin and the loose connective tissue in which it is embedded. The cut section is grayish pink, edematous, but not soft, and microscopically the nodule is seen to consist of masses of endothelial cells, placed close together, lying among capillary vessels and connective tissue fibers. A small number of lymphocytes and polymorphonuclear leucocytes are seen in the interstices of the cutis, but not within the nodule itself. The endothelial walls of the capillaries are thickened, and the lumina of the blood vessels narrowed. Practically every endothelial cell in the nodule contains *Bartonella bacilliformis*, some cells being packed with clumps of organisms. The endothelial cells lining the capillaries are often filled with similar masses of microorganisms. The histological picture at this stage is very simple.

Mature and Vascular Stage.—The interval from the preceding stage to this one varies from a few to 10 to 14 days and persists for 10 days to 2 weeks.

The increase in size of the nodule may be gradual or even relatively rapid until the lesion measures 8 to 10 mm. or more in diameter. The color first becomes bright, then deep, red. The overlying skin is shiny, and the epidermis scaly in places. The nodule is still firm and is sharply demarcated from the surrounding tissues. The color may change to a dark bluish red, suggesting that of a cherry. This type of lesion, especially when deep seated and less vascular on the surface, is characteristic of the form of human lesion known as *verruga nodular*. Occasionally the overlying skin yields to the increasing pressure from within, and the lesion becomes pedunculated.

The lesions found on the scarified lines of the skin are seldom round; on the contrary, they are usually irregular at the surface, and the mass resembles a raspberry in form and color, being made up of a number of small, translucent, red, drupelet-like nodules. The entire region adjacent to the lesion is edematous. The lesion is devoid of an epidermic layer, hence it bleeds readily on slight friction. This type of lesion corresponds to that termed in man *verruga mular*.

In a few animals spontaneous nodules have appeared on the skin at sites remote from those inoculated; these correspond to the general eruption in human cases known as *verruga miliar*. Such lesions have always been small, none becoming larger than 1 to 2 mm. in diameter, but their clinical course is similar to that of the nodules induced by intradermal inoculation or by scarification.

On microscopic examination, nodules or lesions removed during the mature and vascular stage present a somewhat more complex picture than those of the initial stage. The large number of capillary vessels intersecting dense masses of closely packed endothelial cells and the increase of fibrous connective tissue irregularly penetrating the nodular structure are striking features of the lesions. Numerous fibroblasts and fibrils lie between islands of angioblasts, the appearance somewhat suggesting that of a fibrosarcoma. In places there is hyperplasia of the epidermis, and some large detached masses of epithelioid cells find their way deep into the nodular zone, a circumstance which complicates the interpretation of the cellular elements composing the nodule. Some of these invading epithelioid cells show hydropic degeneration. Degenerating endothelial cells and numerous erythrocytes are present in some places and are taken up by migrating macrophages, which also engulf the dead polymorphonuclear leucocytes simultaneously present in these areas. Along the interstitial spaces penetrated by connective tissue fibers, plasma cells and mast cells are sometimes rather numerous. The lumina of the capillary vessels are often occluded or compressed by proliferating endothelial cells. Mitotic figures are common. The skin may be adherent.

The microorganisms are more uniformly present in the endothelial cells along the periphery of the nodule than among the cells in the older foci. None except endothelial cells contain *Bartonella bacilliformis* in the cytoplasm, and no extracellular localization of the parasites can be detected.

When a secondary bacterial invasion has taken place, there are found, needless to say, an enormous number of polymorphonuclear leucocytes loaded with the contaminating bacilli or cocci. At times these bacteria-carrying leucocytes are taken up by endothelial cells and give rise to a confusing appearance, as though the secondary invaders were the primary cause of the hyperplasia.

Regression.—The time of beginning of the regressive process depends on the individual animal's resistance to the infection, and the process of regression is intimately associated with the acquired immunity, local or constitutional. Early regression, before full development of the lesion, has been rather frequent in the experimental animals. Once the nodule has fully developed, however, considerable time is required for its complete resolution, 4 to 5 months in some instances. The first indication of retrogression is the gradual loss of the deep red color. As the color fades the size of the nodule begins to diminish, the paling and diminution continuing until finally only a tiny colorless wart remains. When the nodule ruptures spontaneously at the beginning of the period of regression, the whole mass sloughs and the wound heals rapidly with scar formation. The result is quite different, however, when the nodule is injured, or partially removed by surgical operation during its developing stages; in this instance the lesion acquires renewed vigor and spreads far beyond its previous limits in the form of a *verruga mular;* it becomes malignant.

The histological appearances of the lesions, as just presented, are similar to those observed by the Harvard Commission[7] in human and experimental verruga tissues, of which they carefully studied a large number. The eosin-staining inclusions so constantly encountered in the endothelial cells, both in human and monkey nodules, and particularly emphasized by the Commission, are of considerable significance. These reddish granules, which may have been identical with those described by Mayer, Rocha-Lima, and Werner[8] as Chlamydozoa, in all probability represent intracellular forms of *Bartonella bacilliformis*, somewhat less distinctly stained than in our preparations. The possibility of indistinct staining is one which cannot be ruled out in connection with this difficultly staining microorganism.

[7] Strong, R. P., Tyzzer, E. E., Sellards, A. W., Brues, C. T., and Gastiaburú, J. C., Report of first expedition to South America, 1913, Harvard School of Tropical Medicine, Cambridge, 1915.

[8] Mayer, M., Rocha-Lima, H., and Werner, H., *Münch. med. Woch.*, 1913, lx, 739.

SUMMARY.

Bartonella bacilliformis failed to induce lesions when merely rubbed on the surface of the intact skin of a chimpanzee, an ourang-utan, and numerous *Macacus rhesus* monkeys, although when applied to the scarified skin of the same animals it gave rise to extensive lesions.

Application of infectious material to the scarified skin did not always induce verruga lesions, but intradermal inoculation almost invariably gave rise to nodule formation.

The localization of *Bartonella bacilliformis* in the skin is not, in experimental animals, determined by mechanical factors, since scarification of the skin or intradermal injection of foreign substances in monkeys infected with *Bartonella bacilliformis* does not give rise to verruga formation.

The degree of susceptibility of the skin tissues appears to be considerably diminished during the course of experimental infection with *Bartonella bacilliformis*. Inoculation of the scarified skin of infected animals gave uniformly negative results, and intradermal inoculation induced only a mild local reaction. In a few exceptional instances, however, of animals previously infected with the strain of *Bartonella bacilliformis* derived from a human verruga nodule, reinoculation with the same strain gave rise to unusually marked reactions.

The evolution of the skin lesion induced in experimental animals by *Bartonella bacilliformis* may be divided into four stages, the period of incubation, the initial stage, the mature and vascular stage, and the regression. In the initial stage the lesion is a pure angioendothelioma, but in the stage of full development the histological picture is complicated by connective tissue proliferation and occasionally also by penetration of epidermis into the lesion. The demonstration of *Bartonella bacilliformis* in the endothelial cells distinguishes the lesion from others which simulate it.

The cutaneous lesions known as *verruga nodular*, *verruga mular*, and *verruga miliar* have been reproduced in monkeys.

EXPERIMENTS ON THE PRODUCTION OF WASSERMANN REAGINS BY MEANS OF TRYPANOSOMES.

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According to the original view of the discoverers the Wassermann reaction was considered, in analogy to the common serological reactions, as being caused by antibodies specific for the infecting microorganisms. This view became doubtful and was abandoned by most authors when it was shown that syphilitic sera react with alcoholic extracts of organs, presumably with lipoids present in these extracts (1–3). The use of extracts of normal instead of syphilitic organs (1) has since been accepted almost generally for the technique of the Wassermann reaction. Several main hypotheses have been advanced in order to explain the formation and properties of the Wassermann reagins. Some authors believed that the action of the syphilitic sera was due not to immune bodies but to non-specific changes of the serum proteins. Others regarded the reagins as antibodies formed either by autoimmunization with lipoidal substances from the infected organism or with lipoids derived from the spirochetes (*cf.* Bergel). In the latter case one had to assume that the antibodies produced act not only upon the homologous antigen but also upon lipoids of very different origin.

The hypotheses implying an immunization against lipoids met with the difficulty that the attempts to induce the formation of antibodies by injection of lipoids, as a rule, did not yield clear-cut and consistent results. In the positive experiments recorded there was no certainty as to the absence of small quantities of antigenic proteins in the material injected or else the amount of antibody formation was rather insignificant.[1]

[1] Regarding the antigens of blood corpuscles see "On the antigens of red blood corpuscles. The question of lipoid antigens," by K. Landsteiner and J. van der Scheer, *J. Exp. Med.*, 1925, xli, 427.

A new impulse was given to the study of lipoid antigens by the work on the Forssman's heterogenetic reaction. It led to the view that Forssman's antigen has a complex constitution and is composed of a specific part binding but not immunizing, which part can be extracted by alcohol, and of a protein responsible for the immunizing effect (4–6). Moreover by means of a special method regular results in the immunization with alcoholic extracts of Forssman's antigen (horse kidney) could be obtained (5, 7). These extracts became antigenic when mixed with protein solutions such as human or pig serum, although in themselves they lack this property or exhibit it to a very small degree only. The case of the Forssman antigen is not exceptional as was shown by the application of the same method to the alcoholic extracts of erythrocytes (8). Evidently the findings reported suggested the view that the antigen operative in the production of Wassermann reagins may possibly be of a complex structure analogous to that of the heterogenetic antigen (5, p. 305).

The statement made by Taniguchi (9) that animals treated with Forssman's antigen may develop Wassermann reagins beside the hemolytic antibodies offered no satisfactory explanation since organs free from Forssman antigen did not produce a like effect in his experiments.

Sachs, A. Klopstock and Weil (10) emphasized a hypothesis related to that of Citron. They assume that the change in the serum is due to the action of a combination of lipoids of the tissues with proteins of the spirochetes. The authors actually were able to show that, in analogy to the experiments on heterogenetic antigen, after injections of alcoholic extracts of rabbit organs along with pig serum into rabbits the sera of the animals became Wassermann-positive.

The idea that the microbes contain the antigen responsible for the characteristic alteration of the serum was examined by F. Klopstock (11) and by ourselves (12). In Klopstock's experiments the Wassermann-positive sera were got by injections with killed *Spirochæta pallida*, in ours with killed *Trypanosoma equiperdum*. The use of trypanosomes for the study of the Wassermann reactions is warranted by the fact that infections with trypanosomes often result in the appearance of a positive Wassermann reaction (13, 14) which is

apparently equivalent to that of syphilitic sera. The occurrence of these reactions in rabbits seems to vary considerably with different strains of trypanosomes according to our experience. (See Browning and McKenzie (15).)

The results of our investigation briefly described in a preliminary communication (12) are given more fully in the present paper.

EXPERIMENTAL.

Rabbits were injected with a suspension of dead trypanosomes (*Trypanosoma equiperdum*) prepared as follows: Citrated blood taken from rats at the height of the infection was diluted with an equal volume of saline and the erythrocytes sedimented by gentle spinning. The supernatant fluid and the resuspended white layer on top of the blood corpuscles were joined and the trypanosomes sedimented by vigorous centrifugalization. The sediment was washed once with about 25 times its volume of saline. A suspension was made with about 4 cc. of saline for every rat used. This turbid fluid was rich in live trypanosomes and contained few blood cells when examined microscopically. After addition of one-twentieth of its volume of 5 per cent phenol it was kept in the ice box overnight. By that time the microbes were largely disintegrated. No infections with trypanosomes occurred upon administration of even large doses of this material.

The strain of trypanosomes employed was of low virulence for rabbits, that is, a very chronic disease developed. The lesions, however, were intense and typical.

In all the experiments special care had to be taken in selecting the animals owing to the frequent occurrence of positive reacting sera in stock rabbits. Therefore the sera were tested with the flocculation method and the complement fixation with cholesterolized beef heart extract and with emulsions of commercial egg lecithin (Merck). The animals were used only if these tests were completely negative, or, in some cases, gave very weak reactions with the Sachs-Georgi method with serum diluted 1:2. In order to pick out enough rabbits for the experiments the selection had to be made from a rather great number of animals.

Wassermann Tests.—To 0.25 cc. of progressively doubled dilutions of inactivated serum starting with 1:5 was added 0.25 cc. of an emulsion of cholesterolized beef heart extract and 0.25 cc. of 1:10 guinea pig serum. After incubation for 1 hour at 37°C. 0.25 cc. of diluted sheep blood immune serum containing 2½ to 3 hemolytic units and 1 drop of 50 per cent sheep blood were added.

The antigen was prepared by extracting beef heart with 5 volumes of alcohol 95 per cent and adding 0.06 of the volume of a 1 per cent alcoholic solution of cholesterol. From this an emulsion was made by adding first 1 volume and after about 10 seconds 4 volumes of saline solution.

Sachs-Georgi Tests.—0.2 cc. of the inactivated serum diluted 1:2 and 1:10 were mixed with 0.2 cc. of the emulsion of cholesterolized beef heart extract. The tubes were kept at 37°C. for 2 hours and at room temperature overnight.

TABLE I.

Injections with Dead Trypanosomes. The Animals Were Tested on the Same Day, after They Had Been Injected 4 to 7 Times.

No. of animals	Before injection	After injections with dead trypanosomes						
	Sachs-Georgi flocculation with serum diluted 1:2	Wassermann reaction with cholesterolized beef heart extract	Sachs-Georgi flocculation with serum diluted 1:		Complement fixation with lecithin	Flocculation of lecithin with serum diluted 1:2	Complement fixation with alcoholic extract of trypanosomes	No. of injections
			2	10				
1	0	0,0,tr,vstr	+++	+±	ac,c,c	0	0,0,str,c	5
2	0	0,0,0,d,ac,c	+++	+±	c,c,c	0	0,0,0,str,ac	5
3	0	0,0,0,0,str,c	++++	+++	str,vstr,ac,c	0	0,0,0,0,str	4
4	tr	w,d,str,c*	+*	0	vstr,d,str,ac,c	ftr	0,0,w,ac,c	7
5	0	0,0,0,0,d,c	+++	+++	ac,ac,c	0	0,0,0,tr,c	6
6	0	0,0,0,0,0,str	+++	+++	w,0,w,ac,ac,c	+	0,0,0,0,vstr	4
7	tr	0,0,0,vstr,c	+++	++±	d,str,c	±	0,0,0,str,c	5
8	0	d,w,str,ac,c	++	+	c,c,c	0	0,0,ftr,tr,ac,c	7

* Tests after 7 injections. The reactions with this serum were strongly positive after 4 injections.

Lecithin Tests.—For the complement fixation 1 part of a ½ per cent alcoholic solution of egg lecithin Merck was emulsified by fairly rapid addition of 24 parts of saline solution. The fluid is opalescent. The complement fixation tests were made as described above. This way of preparing the emulsion was found necessary to avoid reactions with normal sera.

For the flocculation tests (with inactivated serum 1:2) the emulsion was prepared by rapid addition of 5 parts of saline solution to 1 part of a ½ per cent

TABLE II.

Sera of Rabbits Infected with Trypanosomes Tested 1 Month after the Infection

No. of animals	Before infection	1 mo. after the infection with trypanosomes					
	Sachs-Georgi flocculation with serum diluted 1:2	Wassermann reaction with cholesterolized beef heart extract	Sachs-Georgi flocculation with serum diluted 1: 2	Sachs-Georgi flocculation with serum diluted 1: 10	Complement fixation with lecithin	Flocculation of lecithin with serum diluted 1:2	Complement fixation with alcoholic extract of trypanosomes
9	0	0,0,0,0,str,c	+++	+±	0,0,0,str,c	++±	tr,d,str,c
10	0	ac,ac,ac,c	+±	0	c,c,c	±	vstr,vstr,ac,ac
11	0	str,str,ac,c	+	ftr	0,0,c	+±	vstr,vstr,ac,ac
12	0	0,0,0,ac,c	+++	+	0,0,ftr,ac,c	++±	tr,tr,str,vstr,c
13	0	str,str,vstr,c	+±	±	w,0,w,ac,ac,c	++	ac,ac,c
14	±	0,0,0,tr,c	++±	+	0,0,0,0,ac,c	++	str,str,vstr,ac,c
15	0	0,0,vstr,c	+	tr	0,0,vstr,c	+±	vstr,ac,ac,c
			Normal Rabbits.				
A		c,c,c,c	0		c,c,c,c	0	c,c,c,c
B		c,c,c,c	0		ac,c,c,c	0	c,c,c,c

The tests recorded in Tables I and II were made on the same day and with the same emulsions. For comparison the reactions of sera of normal rabbits are recorded.

solution of the lecithin. The readings were taken after 20 hours at room temperature.

Tests with Trypanosome Extract.—1 volume of the packed microbes was extracted 24 hours with 25 parts of 95 per cent alcohol. The filtered solution was emulsified by fairly rapid addition of 5 parts of saline solution and the emulsion was used for complement fixation as in the Wassermann tests.

The strength of the reactions in these tests is indicated as follows: Hemolysin tests—0 = no hemolysis, ftr = faint trace, tr = trace, w = weak, d = distinct,

TABLE III.—*8 Rabbits Were Injected with Dead Trypanosomes on April 24, 30, and May 5, May 5*

No. of animals	Before injection					After 2 injections with dead try			
	Wassermann reaction with cholesterolized beef heart extract	Sachs-Georgi flocculation with serum diluted 1:2	Complement fixation with lecithin	Flocculation of lecithin with serum diluted 1:2	Complement fixation with alcoholic extract of trypanosomes	Wassermann reaction with cholesterolized beef heart extract	Sachs-Georgi flocculation with serum diluted 1:		Complement fixation with lecithin
							2	10	
16	c,c,c,c	0	c,c,c,c	0	ac,c,c,c	vstr,ac,c	tr	0	ac,ac,c
17	c,c,c,c	0	c,c,c,c	0	c,c,c,c	0,0,w,c	++±	±	c,c,c
18	c,c,c,c	0	c,c,c,c	0	c,c,c,c	d,str,c,c	+	0	ac,c,c
19	c,c,c,c	tr	c,c,c,c	0	c,c,c,c	vstr,ac,c	±	0	c,c,c
20	c,c,c,c	0	c,c,c,c	0	c,c,c,c	0,0,d,c	+	tr	vstr,ac,c
21	c,c,c,c	ftr	c,c,c,c	0	ac,c,c,c	0,0,0,d,c	+++	++	ac,ac,c
22	c,c,c,c	0	c,c,c,c	0	c,c,c,c	0,0,w,ac,c	+++	+	ac,c,c
23	c,c,c,c	0	c,c,c,c	0	c,c,c,c	d,w,vstr,ac	ftr	0	c,c,c,c
									Normal Rab
C D						c,c,c	0	0	ac,c,c

* Tests 13 days after the 1st injection.

with Doses Corresponding to 6 or 7 Mg. Dry Weight of Trypanosomes. The Tests Were Made on and 13.

panosomes (after 11 days)		After 3 injections with dead trypanosomes (after 19 days)					
Flocculation of lecithin with serum diluted 1:2	Complement fixation with alcoholic extract of trypanosomes	Wassermann reaction with cholesterolized beef heart extract	Sachs-Georgi flocculation with serum diluted 1: 2	Sachs-Georgi flocculation with serum diluted 1: 10	Complement fixation with lecithin	Flocculation of lecithin with serum diluted 1:2	Complement fixation with alcoholic extract of trypanosomes
0	0,w,ac,c	0,0,0,0,0,ftr,ac,c	++	++±	c,c,c,c	0	0,0,0,0,w,vstr,ac
tr	0,0,w,vstr	0,0,0,0,0,0,vstr,ac	+++	+++	c,c,ac,c	0	0,0,0,0,0,w,ac
0	0,tr,vstr,c	0,0,0,0,str,c,c	+++	++±	c,c,c,c	+±	0,0,tr,vstr,c
0	str,vstr,d	0,0,0,0,vstr,c	+++	+±	c,c,c,c	±	0,0,str,ac,c
0	0,0,d,ac,c	0,0,0,0,vstr,c	+±	±	c,c,c,c	0	0,0,d,ac,c
0	0,0,0,str,c	0,0,0,ftr,ac,c	+++	++±	c,c,c,c	0	0,0,0,0,w,c
0	0,0,tr,c	0,0,0,0,0,0,d,ac	+++	+++	c,c,c,c	tr	0,0,0,0,ftr,vstr,c
0	tr,str,c*	str,d,vstr,ac,c	ftr	0	c,c,c,c	0	0,0,0,vstr

bit Sera.

0	c,c,c	c,c,c	0	0	c,c,c	0	c,c,c
		ac,c,c	0	0	c,c,c	0	c,c,c

TABLE IV.

Tests with Sera of 7 Rabbits Infected with Trypanosomes.

The table reproduces the results at the time when the strongest reactions were observed. In the last column the number of days after the infection is given.

No. of animals	Before infection					After infection					
	Wassermann reaction with cholesterolized beef heart extract	Sachs-Georgi flocculation with serum diluted 1:2	Complement fixation with lecithin	Flocculation with lecithin with serum diluted 1:2	Complement fixation with alcoholic extract of trypanosomes	Wassermann reaction with cholesterolized beef heart extract	Sachs-Georgi flocculation 1:2	Sachs-Georgi flocculation 1:10	Complement fixation with lecithin	Flocculation with lecithin with serum diluted 1:2	Complement fixation with alcoholic extract of trypanosomes	Days after infection
24	c,c,c,c	tr	c,c,c,c	0	ac,c,c,c	0,0,0,0,0,str,c	+++	+++	0,0,0,ftr,str,ac,c	+++	tr,ftr,0,w,str	32
25	ac,ac,c,c	tr	c,c,c,c	0	c,c,c,c	0,0,0,ftr,vstr	+±	+	0,0,0,tr,ac,c	+	w,w,d,str,ac	32
26	c,c,c	0	c,c,c	0	c,c,c	vstr,ac,c,c	tr	ftr	ac,c,c,c	0	ac,c,c,c	22
27	ac,c,c	0	c,c,c	0	c,c,c	0,0,0,0,0,vstr,c	+++	++±	0,0,0,0,0,c	+++	0,tr,w,vstr,ac,c	50
28	ac,c,c	0	c,c,c	0	c,c,c	d,tr,str,ac	tr	0	w,str,ac,c	+	d,str,vstr,c	22
29	vstr,c,c	0	c,c,c	0	c,c,c	0,0,0,0,vstr,ac,c	+++	++	0,0,0,str,c	++±	vstr,str,ac,c,c	42
30	c,c,c	0	c,c,c	0	c,c,c	0,0,0,vstr,ac,c	+±	±	0,0,w,ac,c	++	d,w,vstr,ac,c	42

str = strong, vstr = very strong, ac = almost complete, c = complete hemolysis. Flocculation tests—0 = no flocculation, ftr = faint trace, tr = trace; ±, +, ++, +++, etc.

In all experiments positive sera of known strength and negative ones were used as a control.

In our first experiments 7 rabbits were infected by injection of 1 cc., 50 times diluted blood from an infected rat, and 8 rabbits re-

TABLE V.

Human Syphilis Sera.

Nos. of the sera	Wassermann reaction with cholesterolized beef heart extract	Sachs-Georgi tests with serum diluted 1:		Complement fixation with lecithin	Flocculation of lecithin with serum diluted 1:2	Complement fixation with alcoholic extract of trypanosomes
		2	10			
S_1	0,0,0,0,w,vstr,c	++++	+++	ac,c,c,c	±	str,d,str,vstr,ac
S_2	0,0,0,0,tr,d,str	++++	+++	c,c,c,c	+	ac,vstr,ac,c
S_3	0,w,vstr,c	±	0	vstr,vstr,ac,c	±	
S_4	0,tr,ac,c	+++	++	c,c,c,c	+	c,c,c,c
S_5	0,0,0,d,c	++	+	c,c,c,c	+±	vstr,ac,c
S_6	0,0,0,d,c	+++	++	c,c,c,c	+	ac,ac,c

Rabbit Syphilis Sera.

50	0,0,0,w,ac,ac	+++	++±	0,0,d,ac,c	++±	tr,str,c,c
51	0,0,w,ac	+++	±	tr,str,c	±	
52	0,0,w,c	++±	++±	0,tr,str,ac	++	
53	0,ac,c,c	++±	ftr	d,str,c	0	
54	0,0,0,tr,vstr,ac	++++		0,tr,str,ac,c	++±	0,0,tr,vstr,ac
55	0,0,tr,vstr,vstr	+++	+	0,tr,vstr,ac,c	+	ftr,ftr,d,vstr,c

ceived every 4 to 7 days intravenous injections of $3\frac{1}{2}$ cc. of an emulsion of killed trypanosomes, containing 6 to 7 mg. of dry material.

Table I shows that with dead trypanosomes the sera of 7 of the 8 animals turned positive in the Wassermann and Sachs-Georgi reaction. In most cases the reactions were strong already after 3 to 4 injections. In one case the serum was weak in the Wassermann reaction although distinctly positive with trypanosome extract and in the Sachs-Georgi reaction. In the infected animals the

results were less regular, especially as regards the Wassermann tests, while the lecithin reactions were positive in all cases but one (Table II).

The changes in the reaction during the course of the experiments and also the differences between the various sort of antigens will be referred to later.

TABLE VI.—*Rabbits Injected*

No. of animals				
	No. of days after the first injection	11	19	27
	No. of injections	2	3	4
16	Wassermann reaction	vstr,ac,c	0,0,0,0,0,ftr,ac	0,0,0,0,ftr,str,vst
	Sachs-Georgi with serum diluted 1:2	tr	++	++±
	Complement fixation with trypanosome alcoholic extract	0,w,ac,c	0,0,0,0,w,vstr,ac	0,0,0,0,0,ftr,vstr
	No. of days after the first injection	11	19	27
	No. of injections	2	3	[illegible]
17	Wassermann reaction	0,0,w,c	0,0,0,0,0,0,vstr,ac	0,0,0,tr,str,vstr
	Sachs-Georgi with serum diluted 1:2	++±	+++	+++
	Complement fixation with trypanosome alcoholic extract	0,0,w,vstr	0,0,0,0,0,w,ac	0,0,0,0,tr,str
	No. of days after the first injection	11	19	27
	No. of injections	[illegible]	3	4
18	Wassermann reaction	d,str,c,c	0,0,0,0,str,c	0,0,0,tr,vstr,c
	Sachs-Georgi with serum diluted 1:2	+	+++	++++
	Complement fixation with trypanosome alcoholic extract	0,tr,vstr,c	0,0,tr,vstr,c	0,0,0,0,d,ac

A second experiment was performed similar to the first.

The results presented in Tables III and IV agree well with the foregoing. In the experiment with dead trypanosomes 7 out of 8 animals showed strongly positive Wassermann reactions after 3 injections corresponding to 6 to 7 mg. dry weight 19 days after the first

injection. All the sera reacted positively with trypanosome extract. Frequently the reactions started suddenly. With lecithin no complement fixation was observed while in the former experiment a few sera gave weak reactions. Weak or moderate flocculation reactions occurred in some instances with lecithin. The Sachs-Georgi tests

ith Dead Trypanosomes.

34	40	47	53	62
5	6	7	8	9
0,tr,str,ac,c	w,w,vstr,c	ac,c,c	ac,c,c	d,vstr,ac,c
+++	tr	ftr		ftr
		0,d,str,c	0,d,ac,c	tr,str,vstr,c
34	40	47	53	62
5	6	7	8	9
0,0,w,vstr,c	0,0,w,vstr,c	0,tr,str,ac,c	0,tr,str,ac,c	0,d,vstr,c
++++	++±	++±		++
		0,d,ac,c	0,d,ac,c	0,str,vstr,c

ran parallel to the Wassermann reaction. After 2 injections, 11 days from the beginning of the treatment the fixation tests were already positive in 4 out of 8 animals (see Table III) with Wassermann antigen and 6 were positive with alcoholic trypanosome extract. Presumably the reactions with trypanosome extract precede those

TABLE VII.—*Complement Fixation Tests wit*

No. of animals	No. of days after infection.....	33	47	57
9	Wassermann reaction.....	0,0,0,0,vstr,c	0,0,0,0,ac	0,0,0,0,0,str,ac,c
	Complement fixation with extract of trypanosomes..	tr,d,str,c	d,tr,w,d	0,0,0,w,vstr,ac
	No of days after infection.....	33	47	57
12	Wassermann reaction.....	0,0,0,ac,c	0,0,str,ac,c	w,str,vstr,c
	No. of days after infection.....	33	47	57
14	Wassermann reaction.....	0,0,0,tr,c	0,0,0,0,0	0,0,0,0,0,0,d,c
	Complement fixation with extract of trypanosomes..	str,str,vstr,ac,c	d,ftr,0,tr,str	ftr,0,0,0,vstr,ac
	No. of days after infection.....	17	24	32
24	Wassermann reaction.....	vstr,c,c	0,0,0,0,tr,vstr,ac	0,0,0,0,0,str,c
	No. of days after infection.....	17	24	32
25	Wassermann reaction.....	vstr,c,c	0,0,0,0,ac	0,0,0,ftr,vstr
	No. of days after infection.....	9	16	22
27	Wassermann reaction.....	w,d,vstr,ac,c	str,vstr,vstr,ac,c	ftr,ftr,d,ac

Sera of Infected Rabbits at Various Stages.

70	84	98	175	
0,0,0,0,0,ac	0,0,ftr,vstr,ac,c	0,0,0,tr,c	w,d,str	
0,tr,vstr,ac,c	tr,w,vstr,c,c	d,w,ac,c	str,c,c	
70	84	[illegible]	175	
ftr,tr,str,c	d,w,vstr,c	0,0,w,ac,c	0,0,0	
70				
0,0,0,0,0,0,vstr				
0,0,tr,vstr,c				
37	43	59	70	145
0,0,0,0,tr,vstr,c	0,0,0,0,0,str,ac	0,0,0,vstr,c,c	tr,d,vstr,c	c,c,c
37	43	56	70	145
tr,0,tr,vstr,c	d,ftr,str,ac,c	ac,c,c	c,c,c,c	vstr,ac,ac
36	50	125		
0,0,0,tr,ac,c	0,0,0,0,0,vstr,c	c,c,c		

with the heart extract in analogy to the findings of F. Klopstock. Regarding these reactions some doubt may arise as to whether or not they are due to a fixation of complement with substances derived from rat cells, since the presence of small quantities of these in the injected material could not be avoided. Control experiments with rat blood extracts however seem to rule out the possibility that such an effect influences the results essentially.

Of the infected animals one failed to develop reagins while in another the response was feeble. Again the reactions with lecithin are well pronounced, those with trypanosome extract are weak. In some cases the latter became strong at a later stage (Table VII, Animals 9 and 14). Positive reactions with alcoholic trypanosome extract are reported by other authors in trypanosomiasis. In this and in the first set of infections the sera gave a strong agglutination when mixed with a suspension of live trypanosomes. The phenomenon was not closely examined. (Rieckenberg's reaction?)

For comparison some tests with sera of human and rabbit syphilis are given in Table V.

It may be of interest to give examples of the changes in the reactions occurring during the course of the experiments (Tables VI and VII).

One sees from the tables that the sera of the animals which were continually injected and also in the infected ones, the antibodies tend to decrease or disappear after a certain time. In some of the infected rabbits kept under observation for several months this drop was followed by a recurrence of the reactions (for instance Rabbit 12).

In order to ascertain that the effects described are peculiar to the antigens contained in trypanosomes, control experiments were performed with other materials as, cholera vibrios, emulsions of beef and pig kidney, and with pig serum, the same amounts (in terms of dry weight) being used for the injections as in the experiments with dead trypanosomes. In each case 4 rabbits were taken. Only in 1 animal did a distinctly positive Wassermann reaction develop after 4 injections with beef kidney.

DISCUSSION.

The main result of the experiments described is the fact that the sera of rabbits become strongly Wassermann-positive upon injections of dead trypanosomes. Flocculation reactions according to Sachs-Georgi corresponded to the complement fixation.

The immunization was obtained with small quantities of antigen and almost regularly. In this respect the findings resemble those of F. Klopstock with *Spirochæta pallida*. Although injections with other materials may occasionally give rise to positive reactions,[2] a peculiar quality of the microorganisms studied must be assumed in view of the ease with which they induce the production of the reagins. Equal amounts of other substances as vibrios, organs and serum used in control experiments yielded almost uniformly negative results. Also the difference in the effects of injections of normal and syphilitic organs as demonstrated by Citron and Munk (18) and recently confirmed by Heimann (19) can be explained more readily by the action of the microorganisms as such than by the interpretation favored by Heimann.

As mentioned before Sachs, A. Klopstock and Weil attribute the appearance of the Wassermann reagins to an autoimmunization against tissue lipoids bound to proteins of the spirochetes. A certain difficulty arises for the idea of Sachs and his coworkers from the fact that the method employed in their experiments calls for an intensive treatment with large amounts of antigenic material (serum). Furthermore their findings do not explain the occurrence of the Wassermann reaction just in cases of syphilis in contradistinction to other infectious diseases unless one assume, as the authors do, an exceptional aptitude on the part of the spirochetes to bring forth the antigenic properties of lipoids. Such an assumption however has not yet been substantiated by experimental data. At first sight the demonstration of a particular antigenic activity of the spirochetes and trypanosomes themselves seems to give a more natural answer to the question of the formation of Wassermann reagins.

[2] See Brandt, Guth and Müller (16), van der Scheer (17), Klopstock, F., *Deutsch. med. Woch.*, 1925, li, 1701. In the latter experiments organs were used of rabbits which had undergone a treatment with dead bacilli.

To be sure, there still remain several aspects of the problem requiring further explanation.[3] If the action of the Wassermann-positive sera on organ lipoids is supposed to be a heterogenetic reaction brought about by antibodies for lipoids of the microbes one should expect the reaction with the latter to be more intense or at least nearly equal in strength to the former. This is true for the experiments of F. Klopstock, and for ours as far as they were performed with injections of killed trypanosomes. On the other hand the reactions which we obtained with the sera of the infected animals were generally much weaker with trypanosome extracts than with the heart lipoids. While the higher dilution of the trypanosome extract, or the fact that it was not cholesterolized, may possibly account for the weaker reactions it does not explain the difference between the two sorts of experiments, namely infection and immunization with killed microorganisms. Another discrepancy appears in the tests with lecithin (complement fixation and flocculation). These are quite generally much stronger in the case of the infected animals. We found such lecithin reactions also with syphilitic sera of rabbits. Positive lecithin reactions have been recorded by Sachs as a result of the combination method.

Thus one may conclude that the effects are not altogether conformable in both cases; *viz.*, treatment with dead trypanosomes and infection.

In our opinion, these differences do not disprove the hypothesis that the microbes are the active agent in the formation of the reagins. The substances derived from the trypanosomes during the infection and those formed in the disintegration *in vitro* may be somewhat different and it is not improbable that such variations are reflected in the response of the animal. Changes in the method of preparing the antigen may suffice to alter the results, and further investigation along this line is desirable.

Still it cannot be stated as yet that the serological changes occurring during the syphilitic infection are due to one single principle. There is for instance not yet a definite explanation for the occur-

[3] As for instance the observations on flocculation reactions with substances other than lipoids. (*Cf.* Weisbach, W., Wassermannsche Reaktion, etc., Jena, 1924.)

rence of positive Wassermann and flocculation reactions in normal animals.

Mention may also be made of the autohemagglutinins found in infections with protozoa and of autohemolysins (paroxysmal hemoglobinuria), apparently specific (20, 21) for human blood in cases of syphilis, though this phenomenon is rarely met with. Perhaps these observations may be indicative of an autoimmunization.

SUMMARY.

Since it is known that positive Wassermann reactions prevail in trypanosomiasis of rabbits, similar to those in syphilis, trypanosomes were used for an inquiry into the cause of this reaction.

Injections with dead trypanosomes into rabbits proved that these microbes are highly active antigens and suffice in themselves to produce strongly positive Wassermann sera, in analogy to the findings reported by F. Klopstock with *Spirochæta pallida.*

Although a number of questions require further study, yet it seems likely that this antigenic activity of the microbes or their products plays a part in the production of the Wassermann reagins in infections with spirochetes and trypanosomes.

We are indebted to Dr. John A. Kolmer for supplying us with a strain of trypanosomes, and to Dr. Wade H. Brown and Dr. Louise Pearce for the sera of rabbits infected with syphilis.

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INFLUENCE OF LIGHT ON THE GROWTH AND MALIGNANCY OF A TRANSPLANTABLE NEOPLASM OF THE RABBIT.

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One of the striking features of the disease induced by a certain transplantable neoplasm of the rabbit is the variability of its course and of the general character of its manifestations (1). Not only do individual animals differ in respect to the plane or level of malignancy but well defined group variations are also observed between series of rabbits inoculated at one time and those inoculated at another. On the whole, this variability is seasonal in character in that the disease is most severe in the spring and fall and is usually milder in the summer than in the winter months (2). The influence of the seasonal factor has been interpreted as operating upon or affecting general animal economy and susceptibility or resistance to disease is considered as a functional activity of the animal organism.

Although many external and internal factors undoubtedly participate in the seasonal variability of this malignant disease, there appears to be a correlation between sunshine and the manifestations and outcome of the condition. That is, the actual hours of sunshine, together with the rate, extent and persistence of change in the curve of sunshine over a given period, can be related to the observed general level of malignancy. The experiments upon which this relationship has been based were carried out with rabbits kept in rooms which receive practically all light diffused through ordinary window glass so that the effects observed cannot be wholly attributed to the shorter ultra-violet rays.

The idea of a correlation between the external factor of light on the one hand, and the physical state and functional activities of the animal organism on the other, has been tested by a series of experiments in which conditions of light could be controlled. The particular

points studied in beginning the work were the effects produced by a continuous illumination of maximum intensity practicable with simple equipment and by an absence of light, first upon the physical state of normal rabbits as shown by the weights of organs (3) and second, upon the course and character of the malignant disease. The results of the first tumor experiment which were summarized in a preliminary note (4) published soon after the conclusion of the work are here reported in full.

Methods and Material.

The experiment was begun on January 16 and concluded on April 15, 1925. Three animal rooms, with similar environmental conditions other than those of lighting, were employed. These rooms will be referred to as the light, the dark and the control or unaltered room, respectively.

A *constant* source of light was furnished by twelve 1000 watt Mazda lamps and three Cooper Hewitt 50 inch low pressure mercury arcs, Type P, in crown glass, arranged on a rectangular frame in the center of a room from which all other light was excluded. The lamps and arcs were distributed in three horizontal rows 2½ feet apart and each row contained four lamps and one arc. The animal cages were placed parallel with and on either side of the light source at a distance of 3¼ feet.

The intensity of light reaching the cages was not entirely uniform, but on the average was 425 foot candles. The spectrogram of the mercury arc furnished by the Cooper Hewitt Company, shows that the crown glass absorbs all light below a wave-length of 3022-28 Ångström units while that of the Mazda lamps is cut off at about 3100 Ångström units. Our primary object in so far as the quality of the light was concerned, was the absence of rays which are absorbed by ordinary glass, as in the case of the diffused sunlight of the laboratory.

A second room was arranged so that all light was constantly excluded except during the time the animals were fed or examined, when a single 30 watt Mazda lamp was employed. This light was not used for more than 1 hour per day.

An ordinary animal room which has a southern exposure was used as the control room. Practically all the light of this room was diffused sunshine which passed through the glass of two large windows.

The temperature of the three rooms was maintained at 70° to 75°F. by automatic regulation of the common heating and ventilating systems. In the case of the light room, this was supplemented by an electric fan intake of outside air which also served as an aid to satisfactory ventilation. With this equipment, it was not possible to keep the temperature of the light room within the above limits during the warmer months, so that in subsequent experiments to be reported later, the

type of illumination was modified. The humidity of the three rooms varied with that of the outside air.

The rabbits employed were representative of the usual breeds and types commonly used in this laboratory. They were adult male animals, approximately 1 year old, and were assembled from a carefully selected stock 1 week prior to the experiment. During the entire period of observation, each rabbit was separately caged and fed the standard diet of oats, hay and cabbage.

On January 16, 1925, 10 rabbits were placed in each of the three rooms where they remained for the duration of the experiment. On February 16, 1 month after they had been living in an environment of constant light or of constant darkness or in the variable diffused sunlight of the laboratory, each rabbit was inoculated in the right testicle with 0.3 cc. of a tumor emulsion prepared from an actively growing primary tumor. This neoplasm is considered to be of epithelial origin (1). The experiment was terminated on April 15, 2 months after inoculation, at which time all surviving animals were killed by an injection of air into the marginal ear vein. This period was selected upon the basis of previous experience as being sufficiently long to include the majority of deaths due to tumor involvement or to permit recovery to take place.

Detailed records including body weight deteminations were kept covering the clinical course of the disease and each animal received a careful postmortem examination with special reference to the presence or absence of metastases, the number and distribution of metastases, the degree of involvement of different organs and the state of the growth. The distribution of metastatic foci in particular has been used as a basis for estimating the general character of the disease in those cases in which secondary growths were found. The method used involves the calculation of percentage values of the number of tumor sites or foci in terms of the number of theoretical foci as shown by the actual location of metastases in the first twenty generations of tumor animals (5). There are certain obvious objections to this method. For instance, such organs as the liver or kidneys may be markedly involved with numerous tumors or by only a few which destroy little tissue. However, a comparative classification of the disease, whether of high, moderate or low malignancy, may be brought out in percentage terms by an arrangement of metastatic distribution according to the following divisions:

I. Skin and subcutaneous tissue, superficial lymph nodes, muscles, heart and pericardium, bones and bone marrow, glands of internal secretion with the exception of the suprarenals, the spleen and the central nervous system	30	possible foci
II. Lungs and pleura, liver, kidneys and pancreas	5	" "
III. Extensions and implantations to the retroperitoneal and mediastinal tissues, omentum, mesentery and parietal peritoneum	19	" "
IV. Suprarenals and eyes	4	" "

This system of grouping metastatic foci was selected for these reasons:

It has been found, from the study of several hundred rabbits inoculated in the testicle with the tumor, that there is usually a widespread distribution of metastases, extensions and implantations in those animals in which the most malignant disease develops and in which death occurs within 3 to 5 weeks after inoculation. In these instances, tumors may be found in many organs and tissues as in the skin, the superficial lymph nodes, the muscles, the bones and bone marrow, the heart, the spleen, and the endocrine glands as well as in the liver, the kidneys, the lungs, the retroperitoneal and mediastinal tissues and the serous membranes of the abdominal and pleural cavities. In instances of a somewhat less malignant disease, the most conspicuous and frequent metastases are found in the liver, kidneys, lungs and pancreas. A level of still lower malignancy is chiefly characterized by the predominance of extensions of tumor from the primary growth in the testicle to the retroperitoneal and mediastinal tissues and by implantations upon the omentum, mesentery and parietal peritoneum. If death occurs in these animals during the first 2 months after inoculation, the extensions and implantations referred to are found to be of an extreme grade, or, more frequently, some organ such as the kidneys or hypophysis is also involved. Finally, in those animals in which the disease is very mild, metastases may be found only in such sites as the eyes or suprarenal glands which of course are also involved in instances of high malignancy. On the other hand, metastases to the skin, muscles, bones and endocrine gland group practically never occur in cases of low malignancy.

Results.

The results of this experiment consist, first, of the clinical observations in which the general physical condition of the animals, the character of the primary growth and the development of metastases recognizable during life, are of especial interest; second, the mortality rate including the time of death with reference to the time of inoculation; and third, the postmortem observations, particularly as regards the condition of the primary tumor, the animal incidence of metastases and the number, distribution and state of these growths.

As far as could be determined by observation and by body weight, the health of the rabbits kept under the conditions of constant illumination was excellent, and while no outspoken deleterious effects were observed in the animals kept in the dark, there were indications furnished by body weight determinations to the effect that this environment was a less favorable one (Table I). Only the weights of rabbits surviving the experiment have been used in this connection since a loss of weight together with other manifestations of cachexia

frequently occur in association with a widespread tumor growth in animals which succumb to the disease. Reference to the table shows that the groups of rabbits kept in the environment of constant light and of the variable sunlight conditions of an ordinary animal room for 3 months showed a well marked increase in body weight while the group in constant darkness gained comparatively little. Reckoning from the mean weight on admission the controls gained more than the light group due, probably, to the lower initial figure of the controls. However, the final mean weights of both groups were the same. The net gains over the first observations available after the rabbits from the light and dark rooms had been placed in these rooms were practically the same for the light group and the controls, but the dark room group gained only one-third as much as the others.

TABLE I.

Changes in Mean Body Weight.

Group	On admission	Experimental period			Percentage gain	
		1 wk.	4 wks. Day of inoculation	12 wks.	Over admission weight	Over first experimental weight
	gm.	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
Controls.....	2120	2350	2465	2660	25.5	13.2
Lights.....	2264	2342	2428	2675	18.2	14.2
Darks.....	2283	2238	2288	2346	2.7	4.8

A primary tumor developed in each rabbit of the experiment. In the control group,[1] there was a rapid and extensive primary growth for the first 3 weeks, after which time regression took place in the 5 rabbits which survived the experimental period of 2 months, and in 3 of these complete absorption with healing occurred. In the 4 rabbits dying from tumor, there was relatively little necrosis of the primary growth. In the rabbits from the dark room the primary growth was very rapid in 3 animals which early succumbed to the disease, while in 7 rabbits it grew more slowly and ultimately regressed and at the end of the experiment it was entirely necrotic in 2 and

[1] One of the control rabbits died of an intercurrent infection so that this group consists of 9 animals.

healed in 5 instances. In the group exposed to constant illumination, the initial growth of the tumor in the majority of the animals was unusually rapid and extensive but it eventually became entirely necrotic in 5 and completely healed in 4 rabbits. In the remaining animal, which finally succumbed to the disease, approximately one-half of the primary tumor was living.

TABLE II.

Analysis of Results.

Group	No. of rabbits	Metastases found during life		Mortality			Postmortem observations of metastatic foci						Actual and probable recoveries
		Animal incidence	Total No.	No.	Rate	Time after inoculation	Animal incidence	Total No.	Relative rate	Actual rate	No. of foci in		
											Deaths	Survivors	
		per cent			*per cent*	*wks.*	*per cent*						*per cent*
Controls...	9*	44.4	10	4	44.4	4,4, 4.5, 4.5	56.0	81	9.0	16.2	30, 17, 15, 15	4 in 1 rabbit 0 in 4 rabbits	55.6
Darks.....	10	30.0	7	3	30.0	3.5, 3.5, 4	40.0	69	6.9	17.3	26, 25, 17	1 in 1 rabbit 0 in 6 rabbits	70.0
Lights.....	10	20.0	6**	1	10.0	6.5	50.0	27	2.7	5.4	22	5†in 4 rabbits 0 in 5 rabbits	90.0

* 1 rabbit of the original 10 died of an intercurrent bacterial infection during the experiment.

** Two of these metastases eventually became completely healed.

† Three of these metastases occurring in 2 rabbits were small and entirely necrotic.

Metastases in superficial parts of the body which were recognized during life developed in several rabbits (Table II). In the control group there were ten such secondary growths in the eyes, superficial lymph nodes, subcutaneous tissue and bones distributed among 4 rabbits—an animal incidence of 44.4 per cent. In the group kept under conditions of constant darkness, the animal incidence of metas-

tases detected clinically was 30.0 per cent; seven tumors were found in the eyes, lymph nodes, skin and subcutaneous tissue. On the other hand, in only 2 rabbits of the light group—20.0 per cent—were such growths recognized. In 1 rabbit the unusual occurrence of regression and ultimate healing of eye metastases was observed while in another, four tumors were found in the muscles, bones, subcutaneous tissues and superficial lymph nodes.

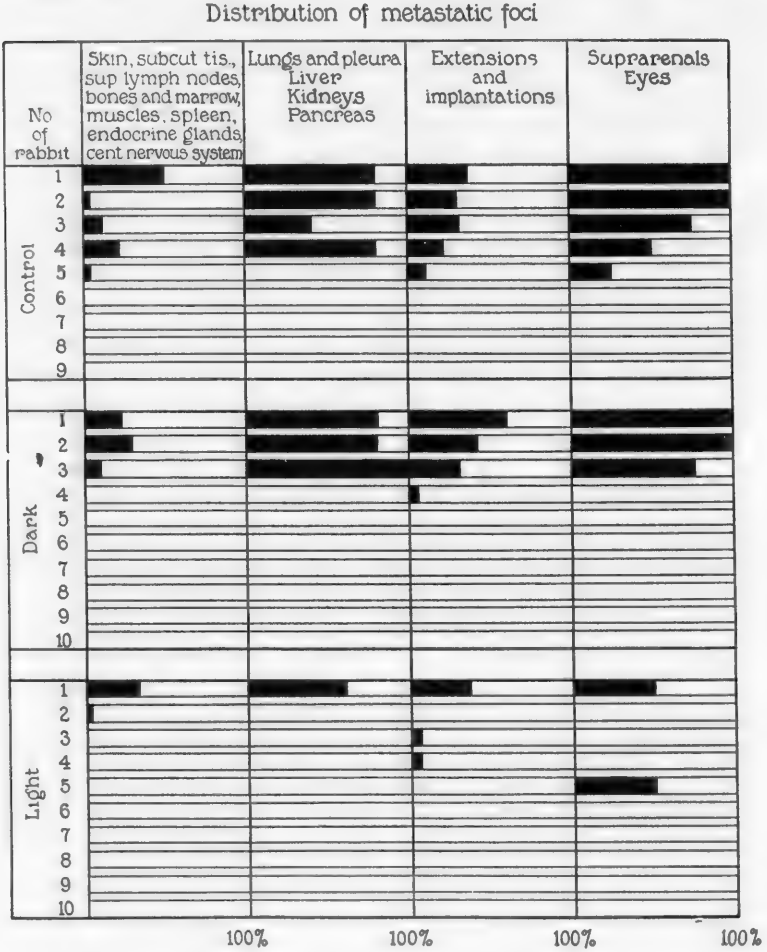
The mortality rates of the three groups were—controls 44.4 per cent; darks 30.0 per cent; lights 10.0 per cent (Table II). Among the controls, 4 rabbits were killed, 4, $4\frac{1}{2}$ and $5\frac{1}{2}$ weeks after inoculation because of the development of paralysis or other symptoms indicative of impending death. A similar procedure was necessary in three instances in the dark room group, $3\frac{1}{2}$ and 4 weeks after inoculation. Among the rabbits in the light room, on the other hand, there was but one such instance. The symptoms which necessitated the sacrificing of this animal $6\frac{1}{2}$ weeks after inoculation, developed much later and more slowly than those of similar cases in either the control or dark room groups.

At postmortem examination, it was found that the incidence of metastases was approximately the same in all three groups, but there were distinct group differences in the number of foci[2] (Table II). Among the 9 control rabbits there were 81 foci of metastatic growths, or 9 per animal, 69 foci in the dark group, or 6.9 per animal, and only 27 foci in the light group, or 2.7 per animal. A comparison of actual metastatic rates in which only rabbits with secondary growths are considered, shows that there was little difference between the control and dark groups, but in the case of the light group the rate was approximately one-third those of the others.

The number of metastatic foci in individual rabbits is given in Table II. In the fatal cases, the largest number occurred in a con-

[2] It should be pointed out that the figures refer to the number of organs or tissues involved, and not to the numbers of secondary growths, and consequently the expressions "foci of metastases," "distribution of metastases," or "metastatic rate" are used rather than "number of metastases." On the other hand, the figures for clinical metastases detected during the life of the animal refer to the actual number of individual secondary tumors found. The present basis of counting foci of metastases is slightly different from that employed in the preliminary note (4).

trol animal in which 30 foci were counted and in the others, there were 15 to 26. But in so far as the severity of the disease may be



TEXT-FIG. 1. Distribution of metastatic foci.

judged from the mean number of metastatic foci, there was practically no difference in the three groups—(controls 19.25, darks 22.7,

lights 22.0). Among the rabbits which survived the experimental period of 2 months, postmortem examination revealed 4 metastatic foci in one control, a single focus in a dark room rabbit and 5 foci distributed among 4 light room animals. In 2 of the rabbits from the light room, the three secondary growths found were small and entirely necrotic. It is probable that none of the 6 surviving rabbits with metastatic foci would have died from the effects of tumor growth.

The character of the disease which developed in the three groups may be compared upon still another, and in many respects a more impressive basis, namely, the distribution of metastatic foci, as is illustrated by Text-fig. 1. In this graph, each rabbit is represented by a horizontal column, divided into four sections corresponding to the four general divisions of secondary growths previously described, while the shading of the sections represents the metastatic rate in percentage terms of the possible number of foci.

This diagrammatic representation illustrates in a striking manner the low plane of malignancy of the disease in the light room group. The metastatic rate in all four divisions is seen to be much lower than that of either the controls or the dark room groups. As far as the dark room series is concerned, the metastatic rate is, on the whole, somewhat lower than that of the controls if one takes into consideration the number of animals with secondary growths and the degree of involvement of the two divisions of foci which are predominately affected in instances of severe or well marked malignancy, namely, the division which includes the skin, muscles, bones and glands of internal secretion, and that of the lungs, liver and kidneys. In the case of the light room group, there was but 1 rabbit in which a disease characterized by a widespread distribution of metastatic growths developed while there were 3 such instances among the dark room and 4 among the control animals. In a 5th control rabbit, there was a wider distribution of tumor than in a 4th dark or in 4 other light room rabbits in which metastases were found and in 2 of these last animals, it will be recalled that the secondary growths were entirely necrotic. Finally, there were 4 controls as contrasted with 6 dark and 5 light room animals in which no metastases were found.

DISCUSSION AND CONCLUSIONS.

This experiment was undertaken for the purpose of obtaining information on the influence of light on the character of disease in rabbits induced by a transplantable malignant neoplasm. To this end, the attempt was made to insure conditions which would be common to all animals of the experiment with the exception of the light environment. Comparable conditions of temperature and humidity prevailed in the three rooms in which the experiment was carried out, all the rabbits were fed the same diet, and the animals themselves, assembled at the laboratory at the same time, were well matched as to breed and age. Individual animal variation could not, of course, be wholly eliminated, but this factor was controlled by the selection and number of rabbits employed in each group.

The lighting of the three rooms was the variable factor of the experiment. One group of rabbits, kept in a well lighted animal room was exposed to the daily fluctuations of sunshine diffused through ordinary window glass. Another group was placed in a room which was continuously and constantly lighted by Mazda lamps and mercury arcs in crown glass. The third group was kept in a room from which all light was constantly excluded except for very brief daily periods.

The clinical and postmortem observations of the three groups of rabbits inoculated with the malignant tumor and kept in these different surroundings bring out distinct variations in the course and character of the tumor process. The most highly malignant disease occurred in the control group living under ordinary indoor daylight conditions. The mortality rate was the highest, the incidence of secondary growths detected during life or found at postmortem examination was also the highest and there was the widest distribution of metastatic foci. In contrast to these effects was the extremely mild disease which developed in the rabbits kept under conditions of constant illumination. The mortality rate of this group was only one-fourth that of the controls and the duration of life of the single rabbit which succumbed to the disease was considerably longer than that of comparable controls. In addition, there was a lower incidence and a smaller number of metastases found during life together with a smaller number of metastatic foci observed at autopsy.

The disease which developed in the group living in practically constant darkness was also not as severe as that of the controls but was considerably more malignant than that of the light room group. As compared with the controls, the mortality rate was slightly lower, the incidence and number of clinical metastases were somewhat less, and there were fewer sites of secondary growths as revealed by postmortem examination. In addition, a higher percentage of rabbits either recovered wholly or was in process of recovery. Although the effect of constant light exclusion was much less pronounced than that of constant and continuous illumination, it appeared to be in the same direction of diminished malignancy.

There were certain special features of the disease that developed in the rabbits kept in the light and dark rooms which have a direct bearing on the influence associated with these environments. The course and character of the primary tumor in the control and dark room groups were in general accord with what is usually observed in the majority of normal rabbits. That is, the tumor underwent a rapid growth during the first 2 weeks and, in animals succumbing to the disease 3 to 5 weeks after inoculation, showed little tendency toward regression while in most rabbits surviving as long as 2 months, it was found to be wholly or largely necrotic or completely healed. In contrast with the control and dark room groups, the initial growth of the primary tumor in the majority of the light room rabbits was unusually rapid and extensive so that the first impression of the disease was one of heightened severity. The subsequent course of the primary growth, however, was in agreement with the low level of malignancy shown by the disease of this group, for it became entirely necrotic or became completely healed in all but 1 rabbit.

Although there was practically no difference in the incidence of metastases in the control and light room groups, the greatly lessened number and the markedly restricted distribution of the growths in the rabbits in the light room, indicate that these animals were able to restrain or inhibit the development of the majority of metastatic foci. This finding is further supported first, by the unusual occurrence in 1 rabbit of the complete healing of the metastatic growths in the iris of both eyes; second, by the postmortem finding in 2 other rabbits of but 3 small and entirely necrotic metastatic tumors and

finally by the comparative lateness with which the terminal stages developed in the 1 rabbit of the group which eventually succumbed to the disease.

The incidence of metastases in the dark room group was slightly lower than in the others. The secondary growths were confined almost wholly to 3 rabbits in which the disease exhibited well marked malignant characteristics, while in the other animals it was extremely mild. These results indicate first, that constant darkness has a slight but definite effect upon the course and character of the disease and second, that this effect differs in some respects from that observed under conditions of constant illumination. It would appear that under the influence of the conditions obtaining in the dark room, those rabbits which possessed a high resistance to tumor growth, either natural or acquired, were able to exercise this power more efficiently but that the influence was not sufficient to enable less resistant animals to deal successfully with the disease. On the other hand, the restraining or inhibitive effect associated with constant illumination was, to a greater or less degree, seen in all rabbits of the group.

The results of this experiment are interpreted as furnishing experimental evidence in support of the idea referred to in the beginning of this paper, that there is a relation between the factor of light and the manifestations and outcome of the malignant disease with which we have been working. It will be recalled that, in general, the periods of maximum and minimum sunlight, corresponding with summer and winter were associated with relatively low levels of malignancy while the periods of greatest malignancy occurred at times of abrupt and rapid changes in the hours of sunshine, coinciding roughly with the spring and autumn months.

In the experiment here reported, the most malignant disease developed in the group of rabbits living in an ordinary room, exposed to the influence of the variations in sunlight, passing through ordinary glass, that prevailed during these months (February, March, April). In contrast with these results, a disease of low malignancy occurred in the group kept in an environment of constant and intense illumination. Furthermore, the plane of malignancy of the disease in the rabbits living in a room from which almost all light was excluded was

found to be somewhat lower than that of the controls although it did not approach the low level of the group exposed to constant illumination. It would appear, therefore, that the conditions of light in the experiment reported were associated with variations in the course and character of the malignant disease comparable with those of a seasonal nature which normally occur throughout the year.

The mechanism by which these effects are induced is not known. We have considered the influence of the seasonal factor in disease as operating upon or affecting general animal economy and susceptibility or resistance as a functional activity of the animal organism. Hence it appears that resistance to tumor growth as displayed by rabbits living under conditions of constant intense illumination and, to a less extent, in almost constant darkness is more potent or efficacious than it is in the case of rabbits exposed to the daily fluctuations of diffused sunlight.

Finally, it should be stated that no attempt was made in this experiment to produce ideal conditions for diminishing or enhancing tumor malignancy. The purpose was to determine whether variations in the course and character of the malignant disease took place, first, under conditions of constant and continuous illumination in which none of the shorter ultra-violet rays participated and, second, under conditions of constant darkness.

SUMMARY.

An experiment is reported in which an environment of constant and continuous light excluding the shorter ultra-violet rays, and one of constant darkness, have influenced the course and character of a malignant disease of rabbits induced by a transplantable neoplasm.

Under the influence of constant light the level of malignancy was observed to be low; under the influence of constant darkness the level of malignancy was somewhat lower than in the control animals living under ordinary indoor light conditions, but the level was not as low as among the animals constantly illuminated.

These observations furnish experimental evidence in support of the idea that there is a correlation between the external factor of light on the one hand and the manifestations of an experimental malignant disease on the other.

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THE INFLUENCE OF LIGHT ON THE REACTION TO INFECTION IN EXPERIMENTAL SYPHILIS.

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The occurrence of wide variations in the character and course of the disease produced in rabbits by inoculation with a given strain of *Treponema pallidum* was recognized long since by us as one of the outstanding features of the experimental infection and we have emphasized the fact that the more decided variations show a tendency to a seasonal periodicity with the occurrence of periods of maximum severity at some time during the spring and fall (1, 2). A survey of available data bearing on this subject seemed to indicate a relation between the course of infection and prevailing meteorological conditions (3). This was particularly true of sunlight expressed in terms of the hours of sunshine over a given period of time. But, the data at hand were not sufficient to indicate the precise nature of the relation that obtained or to warrant a definite conclusion as to the relative importance of the several factors that might be concerned in the production of these effects. It seemed, however, that we had to deal with a general rather than a specific problem, or, that the effects noted in the case of syphilis were incidental to the occurrence of profound changes in the animal organism, influenced in some way by physical environment (3).

With a view to obtaining further information on this subject, several lines of investigations were undertaken. One of these was a systematic study of the normal animal organism in relation to physical environment; another assumed the direction of consecutive observations on the course of syphilitic infections in comparable groups of animals over long periods of time, while a third series of investigations was concerned with direct tests of the effects of physical influences on the reaction to infection. Some of the results of these investigations

have been reported (4, 5). The object of the present paper is to report the results of a series of experiments dealing with the influence of light on the reaction to syphilitic infection. These experiments were undertaken for the purpose of determining, first, whether the course of disease could be influenced by variations in the light environment of animals, comparable to those that occur in consequence of changes in season or weather, and, second, something as to the nature of the effects that might be produced by changes in environment or conditions of this general order. We were, of course, concerned with conditions that might prove to be favorable or unfavorable, but in this instance no effort was made to adjust experimental conditions from this point of view lest by so doing, we might defeat the main object of the experiments. The indications were that at the outset extreme conditions should be employed.

EXPERIMENTAL.

Methods and Material.

The results to be reported represent a comparison of the course of infection in three groups of rabbits living under conditions which, as far as possible, differed only with respect to their light environment. One group of animals, which may be designated as the controls, was kept in a well lighted, well ventilated room with a southern exposure. The temperature of the room was automatically regulated and maintained at a level of 70° to 75°F. while the humidity varied with that of the outside air. The light received by these animals was sunlight which for the most part was filtered through window glass; the amount and quality of the light were determined by natural circumstances.

A second group of rabbits was kept in a room from which all sunlight was excluded; otherwise, the conditions were the same as those of the first room. These animals lived in total darkness except for a brief period each day when it was necessary to use a small Mazda lamp while cleaning cages, feeding, and carrying out routine examinations. For convenience these animals will be referred to as the dark group.

The third group of animals, which may be designated as the light group, was kept in a room similar to the others with sunlight excluded. This room was lighted continuously by 13 Cooper Hewitt, low pressure, mercury arc lights in crown glass (Type P, 50 inches long). The light supplied by these lamps has a wave-length ranging from 3022 to 5790 Ångström units with the greatest concentration between 3650 and 5790. The lamps were mounted on a rectangular frame extending down the middle of the room and were distributed so as to equalize the light which reached cages in a plane parallel with the frame on each side of the

room at a distance of approximately 3½ feet. With this arrangement of lights, an illumination was maintained which amounted on an average to 200 foot candles, being slightly higher opposite the center of the frame than elsewhere. To insure proper ventilation and as an aid in controlling the temperature, a special system of ventilation was used in this room.

The conditions compared were, therefore, (1) intermittent and irregular exposure to diffuse, filtered sunlight of variable quality and intensity, (2) complete exclusion of light, and (3) continuous exposure to artificial light of a given quality and intensity.

Three experiments were carried out for the purpose of determining the effects of these conditions on the reaction of rabbits to syphilitic infection. In each case, 30 male rabbits were used. They were placed in individual cages and divided into three groups of 10 animals each, as nearly comparable as possible with respect to age, breed, and weight. The stipulated conditions of the experiments differed in only one respect, namely, the length of time animals were kept under the conditions described prior to inoculation. In the first experiment, the period was 2 weeks, in the second 4 weeks, and in the third 6 weeks. There were, in addition, differences referable to conditions that prevailed at the time successive experiments were carried out. These are of especial interest in connection with the results obtained in control animals. It is sufficient to say that the first group of animals was inoculated in October, the second in November, and the third in December, 1925. The period covered by the three experiments was, therefore, from October 1, 1925, to April 9, 1926.

All animals were inoculated in one testicle with the Nichols strain of *Treponema pallidum*. The emulsions used were prepared from actively growing testicular lesions and contained from 1 to 3 spirochetes to the microscopic field. Each animal received 0.2 to 0.3 cc. of the emulsion, the exact amount depending upon the spirochetal content. Animals in a given experiment received equal amounts of the same emulsion and the order of inoculation was the same in all cases, namely, controls, light, dark.

The period of observation following inoculation was 4 months, giving a total period of observation of 4½, 5, and 5½ months respectively for the first, second, and third experiments.

In these experiments, special attention was given to the time and frequency of occurrence and to the duration of successive phases of the reaction to infection with a view to reducing the comparison of results to as accurate a basis of quantitative measurement as possible. The particular conditions chosen for comparison were, (1) the incubation period of primary lesions, (2) the time and frequency of occurrence of a critical edema in the inoculated testicle, (3) the time and frequency of occurrence of lesions in the uninoculated testicle (metastatic orchitis), (4) the time and frequency of occurrence of generalized lesions in the skin and mucous membranes, bones, or eyes, (5) the number of foci affected by such lesions, and (6) the proportion of animals that showed complete healing of all lesions during the period of observation (4 months).

For the most part, the methods employed in recording results (Tables I and II and Text-figs. 1 and 2) require no explanation. The term "focal distribution" or "focal incidence" as applied to generalized lesions refers to the number of discrete foci at which lesions developed as determined by actual count. The figures for actual distribution are the mean values for those animals of a group that actually developed generalized lesions while the figures for relative distribution give the results in terms of the entire group. This distinction is made in order to permit comparisons of the extent of the lesions irrespective of the number of animals affected and at the same time to avoid any erroneous impression that might arise from the chance occurrence of an occasional case of severe syphilis in any group of animals.

In Table I and Text-figs. 1 and 2 the results for focal distribution are given in absolute numbers; all other results are in per cent.

Results.

The results of the experiments are recorded in Tables I and II, and Text-figs. 1 and 2. It will be noted that the figures given in Tables I and II represent group values; the detailed results are given in Text-fig. 1 which shows the entire sequence of events from the occurrence of the first primary lesion to the development of the last generalized lesion.

TABLE I.

Incidence of Various Phenomena of Infection and Focal Distribution of Generalized Lesions Actual (F. D. Act.) and Relative (F. D. Rel.).

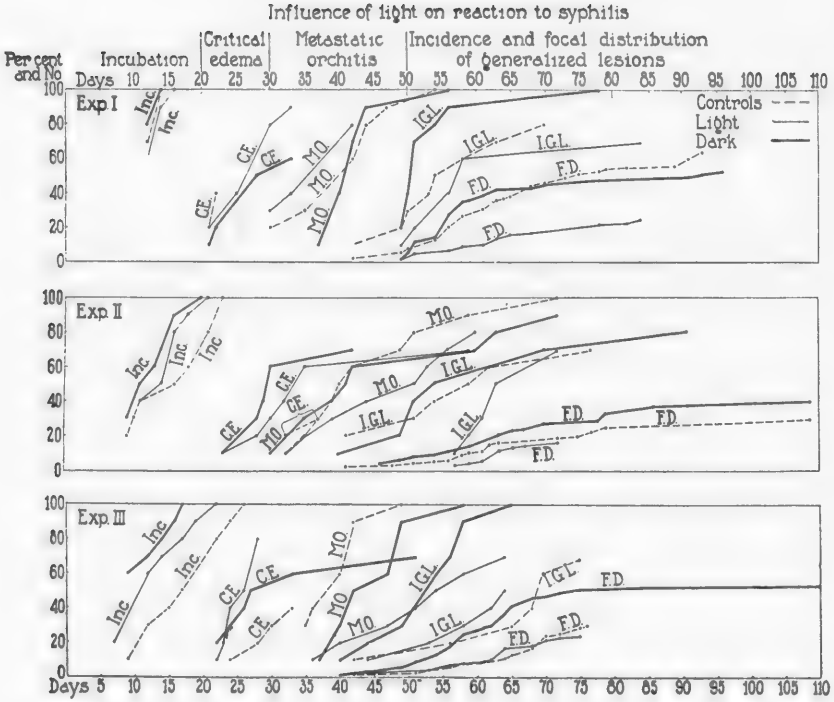
Exp.		Primary lesions	Edema of inoculated testicle	Metastatic orchitis	Generalized lesions		
					Incidence	Focal distribution	Focal distribution
		per cent	*per cent*	*per cent*	*per cent*	*actual*	*relative*
I	C.	100.0	40.0	100.0	80.0	8.0	6.4
	D.	100.0	60.0	100.0	100.0	5.3	5.3
	L.	100.0	90.0	80.0	70.0	3.6	2.5
II	C.	100.0	30.0	100.0	70.0	4.1	2.9
	D.	100.0	70.0	90.0	80.0	5.0	4.0
	L.*	100.0	77.7	88.8	77.7	2.3	1.6
III	C.	100.0	40.0	100.0	70.0	4.1	2.9
	D.	100.0	70.0	100.0	100.0	5.3	5.3
	L.	100.0	80.0	70.0	50.0	4.8	2.4
Mean values	C.	100.0	36.6	100.0	73.3	5.4	4.1
	D.	100.0	66.6	96.6	93.3	5.2	4.9
	L.	100.0	82.5	79.6	65.9	3.6	2.2

* One animal in this group died at the beginning of the experiment; there was also 1 death in the control group and 1 in the dark at the termination of the first experiment.

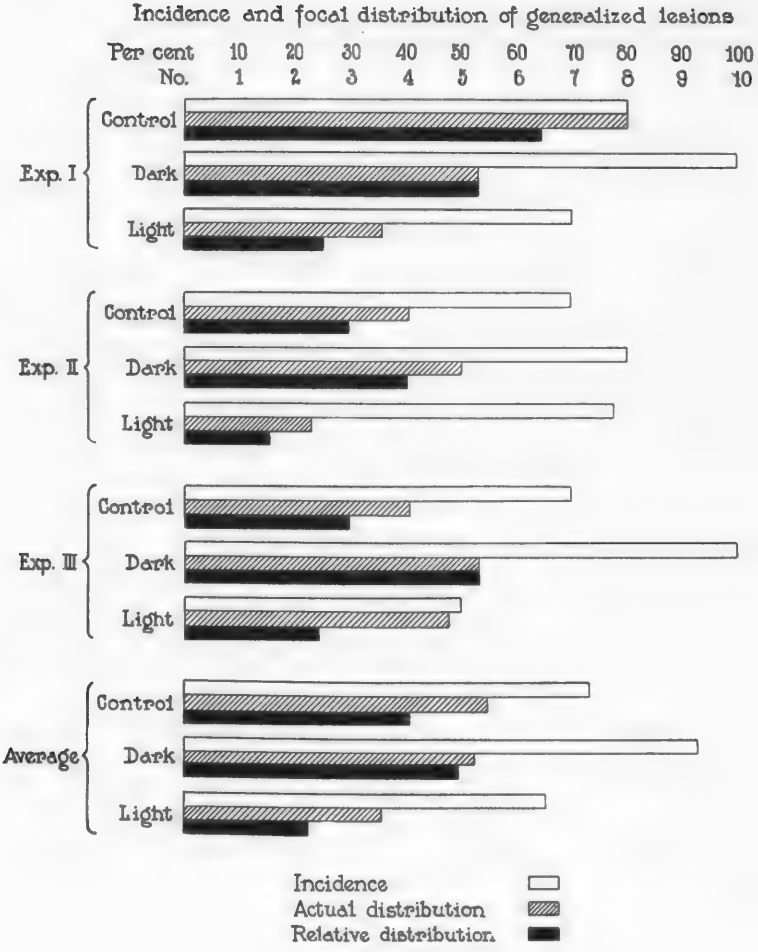
TABLE II.

Mean Time of Occurrence of Various Phenomena of Infection Estimated in Days from the Date of Inoculation.

Exp.		Incubation	Edema	Metastatic orchitis	Generalized lesions		
					First	Last	Mean of all
		days	*days*	*days*	*days*	*days*	*days*
I	C.	12.6	21.5	41.0	54.9	82.4	65.5
	D.	12.4	26.6	42.7	53.9	76.4	60.4
	L.	13.0	27.2	36.4	58.8	73.4	65.2
II	C.	16.1	33.7	45.8	55.0	70.3	69.4
	D.	12.8	30.0	46.6	59.5	84.1	67.1
	L.	14.4	34.6	46.3	63.9	66.4	63.1
III	C.	18.1	28.8	39.6	63.7	72.4	65.0
	D.	11.3	29.3	45.3	53.1	72.4	61.1
	L.	15.2	25.4	50.1	56.2	65.0	61.1
Mean values	C.	15.6	28.0	42.1	57.9	75.0	66.6
	D.	12.2	28.6	44.9	55.5	77.6	62.9
	L.	14.2	29.1	44.2	59.6	68.3	63.1


Influence of light on reaction to syphilis
Per cent and No
Incubation
Critical edema
Metastatic orchitis
Incidence and focal distribution of generalized lesions
Days 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110
Exp I
Exp II
Exp III
Controls
Light
Dark
Inc
CE
MO
IGL
FD
Days 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110

TEXT-FIG. 1.


Incidence and focal distribution of generalized lesions
Per cent 10 20 30 40 50 60 70 80 90 100
No. 1 2 3 4 5 6 7 8 9 10
Exp. I
Control
Dark
Light
Exp. II
Control
Dark
Light
Exp. III
Control
Dark
Light
Average
Control
Dark
Light
Incidence
Actual distribution
Relative distribution

TEXT-FIG. 2.

DISCUSSION AND CONCLUSIONS.

In analyzing the results of the experiments, the most important point to be determined is whether the light environment of the three groups of animals produced any significant difference in their reaction to infection. The effect of any particular condition on the course of disease is of subsidiary importance. The simplest method of approaching this problem is by a comparison of the results obtained for various reactive phenomena in the order of their occurrence, bearing in mind the relations that obtain and the general principles that govern the evolution of syphilitic infections (6).[1] Moreover, the discussion may be limited almost entirely to a consideration of values obtained for group means as the results given by a more detailed analysis of the behavior of individual animals or a consideration of the character of the lesions produced lead to exactly the same conclusions.

Incidence of Primary Lesions and Length of Incubation Period.—All animals in these experiments developed primary lesions but there were some suggestive differences in the incubation period. In the first experiment (Table II, and Text-fig. 1), the incubation period was essentially the same for all three groups of animals with the controls occupying a position intermediate between those in the light and those in the dark. In the second and third experiments, the controls showed a progressive prolongation of the incubation period which amounted to approximately 40 per cent. The animals exposed to artificial light displayed a similar tendency but the differences were less. In the case of the animals in the dark, the incubation period was remarkably constant and such differences as occurred were in the direction of a reduction rather than a prolongation of the time. Combined results for the three experiments, with an average of 15.6 days for the controls, 14.2 for the light, and 12.2 days for the dark group, give a fair index of the promptness of the primary reaction to infection in the three groups of animals.

[1] In addition to the relations that are discussed in the paper referred to (6), it should be noted that there is a basic tendency to the preservation of a uniform interval of time between the occurrence of successive reactions in syphilitic rabbits. With highly virulent strains of *Treponema pallidum* the interval, or reaction time, is approximately 2 weeks and any decided departure from this basic value may be regarded as significant.

Critical Edema.—The occurrence of a critical edema in an inoculated testicle or in association with any other syphilitic lesion is a very variable but highly significant phenomenon. It is indicative of an intense reaction and usually marks the termination of a local reaction, either temporary or final (7).

In the present instance, there was a very striking and constant difference in the frequency with which edema occurred in the three groups of rabbits. The differences are well represented by the combined results for the three experiments which give 36.6, 66.6, and 82.5 per cent respectively for the control, dark, and light groups.

The figures given in Table II indicate a closer agreement with respect to the time at which the edema occurred. In the case of both the control and light groups, the interval between inoculation and the development of edema varied considerably from one experiment to another but remained fairly constant for animals in the dark. If, for the purpose of measuring the progress of the reaction, the time, in days, is estimated from the appearance of the primary lesions, instead of the date of inoculation, the results are slightly different:

	Control	Dark	Light
Exp. I	8.9	14.2	14.2
" II	17.6	17.2	20.2
" III	10.7	18.0	10.2
Mean	12.4	16.5	14.9

Still, these figures, as well as those given in Table II, are somewhat misleading. By reference to Text-fig. 1, it will be seen that, with the the exception of 1 animal each in the light and dark groups of the second experiment and 1 in the dark group of the third experiment, the time of occurrence of edema in individual animals was fairly uniform. The delay in the occurrence of the reaction in the 3 animals referred to overshadows the group tendency so that if the figures are corrected by the omission of these animals, we obtain the following results:

From Inoculation.

	Control	Dark	Light
Exp. I	21.5	26.6	27.2
" II	33.7	26.0	28.5
" III	28.8	25.5	25.4
Mean	28.0	26.0	27.0

From Incubation.

	Control	Dark	Light
Exp. I	8.9	14.2	14.2
" II	17.6	13.2	14.1
" III	10.7	14.2	10.3
Mean	12.4	13.9	12.9

A comparison of results on this basis shows a remarkable constancy in the occurrence of edema among the animals in the light, as well as those in the dark, with a possible tendency toward a shortening of the reaction time. The differences in mean time for the three experiments are comparatively slight, but they are of interest as measures of the progress of the reaction to infection. For example, it will be noted that the time from inoculation to the occurrence of edema in the animals in the dark was shorter than in the controls or the light group, due largely to differences in the incubation period, whereas the time from the development of primary lesions was longer. These differences are suggestive of differences in the efficiency of the reactive mechanism which become more apparent in subsequent reactions.

The most important points brought out by the analysis of the results with respect to the occurrence of a critical edema, are, therefore, the high incidence of edema and the constant time relations shown by both the animals in the dark and those in the light as compared with the much lower incidence and irregular reaction time of the controls.

Metastatic Orchitis.—The development of lesions in an uninoculated testicle occurs with such regularity in rabbits infected with virulent strains of *Treponema pallidum* that the failure of such lesions to occur

may be regarded as evidence of an unusually effective suppression of the infection by some preceding reaction. Variations in the time of development of metastatic lesions are apt to occur and in general the time of occurrence bears a direct relation to the efficiency of preceding reactions.

The outstanding feature of the results as regards the occurrence of metastatic orchitis is the diminished frequency of such lesions in the light animals as compared with the two other groups (Table I). This variation from the expected course occurred in all three experiments but was most decided among those animals that had been exposed to artificial light for the longest time.

The time relations of the reaction are more difficult to interpret. The mean values for the three experiments (Table II) show a comparatively close agreement despite the differences obtained in the first and third experiments and the very irregular development of lesions shown by all groups of animals in the second experiment (see Text-fig. 1).

The situation is clarified somewhat by comparing time intervals (in days) from the development of primary to metastatic lesions and from the occurrence of edema to metastatic orchitis as follows:

From Development of Primary Lesions.

	Control	Dark	Light
Exp. I	28.4	30.3	23.4
" II	29.7	33.8	31.9
" III	21.5	34.0	34.9
Mean	26.5	32.7	30.1

From Occurrence of Edema.

	Control	Dark	Light
Exp. I	19.5	16.1	9.2
" II	12.1	16.6	11.7
" III	10.8	16.0	24.7
Mean	14.1	16.2	15.2

This analysis shows that as we narrow down our basis of reckoning from the rather remote event of inoculation to the occurrence of the reaction immediately preceding the development of metastatic orchitis, relations that are more or less obscure become clearly defined. It is at once apparent that conditions that determined the time of development of metastatic orchitis were not the same in any two groups of animals. The light group and the controls are diametrically opposed whereas the animals in the dark maintain a constant relation from one experiment to another just as they did in the development of primary lesions and edema. In view of what has been said with respect to the significance of the time relations of the reaction, it is important to note that the animals exposed to artificial light showed a progressive prolongation of the reaction time.

Generalized Lesions.—The occurrence or non-occurrence of generalized lesions, the character and extent of the lesions, the time of their appearance, the duration of the period of active development, and the persistence of the lesions afford the most acceptable basis for estimating the effectiveness of the reaction to syphilitic infection in the rabbit. As is well known, the course of disease is subject to wide variation in all of these respects, but, as a rule, is comparatively constant under a given set of conditions.

With respect to the incidence of generalized syphilis, the figures given in Table I show that the condition was definitely more frequent among the animals in the dark and slightly less frequent among those in the light than among the controls. It will be noted, however,that there were fewer cases of generalized syphilis among the control animals of the second and third experiments than in the first and a marked reduction in the number of foci affected (focal distribution, Table I). The animals in the light also showed a reduction in the incidence and distribution of lesions which followed a slightly different order but those in the dark showed very little variation in either of these respects. The relations that obtained are shown graphically in Text-fig. 2.

The apparent reduction in the incidence and distribution figures for the dark group of animals in the second experiment is probably due to a change in the character of the lesions. In the first experiment, the majority of lesions in all three groups of animals were comparatively large and of a destructive character. This was especially

true of controls and of animals in the dark, and in both of these groups there were several cases of unusually severe generalized syphilis. Among the light animals, however, the lesions were smaller, less destructive, and of shorter duration.

There was no material change in the character of the lesions presented by animals of the control and light groups in the second and third experiments. Among the animals in the dark, however, the character of the disease changed. The first lesions tended to be small and of short duration, but were prone to relapse and some of the recurrent lesions attained a large size. This peculiarity of the reaction was at first confusing and, in the second experiment, it was not until some of the lesions had recurred a second or perhaps a third time that the significance of the condition was fully appreciated and a definite diagnosis of generalized syphilis was made. It is probable, therefore, that the incidence and distribution figures for these animals in the second experiment are too low and it is certain that the time given for the occurrence of the first generalized lesions is too long; hence, some allowance should be made for this error in the calculations which involve the time of occurrence of these lesions. The values given for the third experiment are little if at all affected by this factor.

The time relations of the reaction to generalized infection may be viewed in a number of ways. The figures given in Table II show no striking difference in the time from inoculation to the development of generalized lesions in the three groups of animals. The relations change from one experiment to another, but one is impressed chiefly by the closeness of the agreement and the narrow limits of variation. The mean results for the three experiments suggest a precocious development of lesions among the animals in the dark and perhaps a retarded development in the light group if we consider the time at which the first generalized lesions appeared, that is the onset of the generalized reaction, as shown by the following tabulation:

	Control	Dark	Light
Exp. I	54.9	53.9	58.8
" II	55.0	59.5	63.9
" III	63.7	53.1	56.2
Mean	57.9	55.5	59.6

If we take the mean time of development of all generalized lesions, the situation is slightly different. The results in this case are as follows:

	Control	Dark	Light
Exp. I	65.5	60.4	65.2
" II	69.4	67.1	63.1
" III	65.0	61.1	61.1
Mean	66.6	62.9	63.1

Considered from this point of view there is a suggestion that generalized lesions developed earlier on the whole in both the dark and light groups than in the controls.

The results as to the duration of the period of active secondary eruption are more conclusive. The mean time of appearance of the last lesions in each animal was as follows:

	Control	Dark	Light
Exp. I	82.4	76.4	73.4
" II	70.3	84.1	66.4
" III	72.4	72.4	65.0
Mean	75.0	77.6	68.3

This definitely indicates an earlier termination of the period of secondary reaction in the animals of the light group and a probable prolongation of the period in the dark group. Greater differences are shown, however, by a comparison of the mean time between the development of the first and the last lesions in all animals of the three groups as indicated below:

	Control	Dark	Light
Exp. I	27.5	22.5	14.6
" II	15.3	24.6	2.5
" III	8.7	19.3	8.8
Mean	17.2	22.1	8.6

These figures show not only a difference in the character of the reaction, but they furnish an excellent index of the efficiency of the reaction developed by the three groups of animals.

There is one other comparison that may be made for the purpose of bringing out an important relation between the occurrence of generalized lesions and metastatic orchitis. The time interval between these reactions may be estimated from the appearance of the first generalized lesions or from the mean time for all lesions. The respective results are:

From Metastatic Orchitis to Time of First Generalized Lesions.

	Control	Dark	Light
Exp. I	13.9	11.2	22.4
" II	9.2	12.9	17.6
" III	24.1	7.8	6.1
Mean	15.7	10.6	15.4

From Metastatic Orchitis to Mean Time for All Generalized Lesions.

	Control	Dark	Light
Exp. I	24.5	17.7	28.8
" II	23.6	20.5	16.8
" III	25.4	15.8	11.0
Mean	24.5	18.0	18.9

The results obtained by these comparisons show that, while in successive experiments the time between the occurrence of metastatic orchitis and the initiation of the reaction to generalized infection in the control animals was irregular, the mean time between the two reactions was virtually constant. This was not true of either of the experimental groups. The initial time between the two reactions was affected in precisely the same manner and, while from one experiment to another the animals in the dark displayed less variation than those in the light, both showed a progressive reduction of the reaction time. That is, the longer the animals had been exposed to a given condition, the shorter the interval between the development of metastatic orchitis and generalized lesions.

These results are of interest on account of their bearing on the question of the efficiency of the reaction displayed. In the case of animals in the dark, the significance of the results is at once apparent. The short interval between the development of the two sets of lesions was associated with a high incidence of metastatic orchitis and generalized lesions and is to be regarded, therefore, as evidence of an ineffectual reaction. But, the occurrence of a similar reduction in the reaction time of the light animals was associated with two unusual conditions, namely, absence of infection in the uninoculated testicle of some animals and delayed development of lesions in others; in addition, the incidence of generalized lesions was low. It is evident, therefore, that the efficiency of the reaction displayed by these animals was of a higher order, in fact, sufficiently potent to disturb the usual course of events to such an extent as to cause a gradual overlapping of metastatic orchitis and generalized lesions (see Text-fig. 1) but not sufficient to entirely prevent the occurrence of either of these conditions. In the end, generalized lesions actually increased in proportion to the suppression of the reaction in the uninoculated testicle due undoubtedly to the failure of the protection usually afforded by such reactions, hence, the reaction time in the light animals bears an inverse relation to the suppression of lesions in the uninoculated testicle or to the inhibitory effect of the reaction in the inoculated testicle.

This result is not uncommon with strains of *pallidum* of low virulence but is very unusual with strains of high virulence, as under ordinary circumstances, the reaction in the inoculated testicle is rarely sufficient to prevent or materially delay the occurrence of metastatic orchitis. The infection progresses virtually unimpeded until lesions develop in both testicles and not until then does the reaction become sufficiently potent to prevent the occurrence of other lesions. It is evident, therefore, that the primary reaction in the light animals was unusually effective and the same may be said of the secondary reaction as the incidence of generalized lesions was comparatively low and the duration of the period of active development was unusually short.

Recovery.—A final comparison of the behavior of the three groups of animals may be made on the basis of the proportion of animals of each group that showed complete resolution and healing of all lesions

within the period of 4 months' observation. The results, expressed in per cent, were as follows:

	Control	Dark	Light
Exp. I	30.0	50.0	70.0
" II	10.0	50.0	40.0
" III	50.0	40.0	30.0
Mean	30.0	46.6	46.6

Here, again, we find that the results for the controls are irregular while those for the animals in the dark are fairly uniform and the light animals show a progressive reduction in the percentage of recoveries with mean values for both the light and dark groups that are higher than those for the controls. The peculiar feature of these results is the diminishing percentage of recoveries among the light animals which is attributable to a combination of circumstances analogous to those that were pointed out in the discussion of the relations between metastatic orchitis and generalized lesions; that is, an early partial suppression of the infection with consequent delay in the development of full immunity.

Some additional evidence as to the influence of the light environment may be adduced from the records of weight which give a fair index of the physical condition of the three groups of animals during the period covered by the experiments. These results may be tabulated in the form of percentage gains in weight from the beginning to the conclusion of the experiments.

	Control	Dark	Light
Exp. I	21.4	33.5	39.8
" II	38.1	25.6	53.9
" III	31.2	34.1	43.3
Mean	30.2	31.1	45.7

These results are of essentially the same order as those obtained for various phases of the reaction to infection. In fact, the resemblance is so close that one can hardly escape the conclusion that the two sets of results represent closely related effects of a common cause.

The interpretation of results will be facilitated by calling attention to one other feature of the experiments, namely, the differences in the severity of the disease shown by control animals of the three experiments in relation to prevailing conditions of sunlight. By reference to tables and text-figures, it will be seen that, in the first experiment, the infection was much more severe than in the second or third. The comparative results for incidence and distribution of generalized lesions were:

	Incidence	Actual distribution	Relative distribution
Exp. I	80.0	8.0	6.4
" II	70.0	4.1	2.9
" III	70.0	4.1	2.9

The monthly hours of sunshine (Central Park Observatory, New York) for the period immediately preceding and covered by the experiments were:

	Mean normal	Actual	Departure from normal
August	272.3	316.0	+43.7
September	230.4	210.4	−20.0
October	200.8	193.3	−7.5
November	152.6	184.0	+31.4
December	140.2	168.2	+28.0
January	139.7	161.8	+22.1
February	158.5	164.3	+5.8
March	203.7	274.2	+70.5
April	227.7	280.9	+53.2

The points of interest brought out by these figures are, first, the marked reduction in sunshine immediately preceding and following the inoculation of the first group of animals (Experiment I), second, the (unusually) high value for November, and, third, the maintenance of an almost constant and unusually high level of sunshine for the months of December, January, and February which covers the critical period of the second and third experiments. As will be seen, the actual level of sunshine for the 3 months was slightly higher than the normal November and February level or represented a mid-fall and early spring rather than a winter condition.

Without attempting to analyze these relations in detail, it may be pointed out that the occurrence of the most severe infection followed an abrupt and decided change in the prevailing level of sunshine and that, with the stabilization of the sunshine curve at a slightly lower level, the infection assumed a milder form; there were irregularities in the progress of the disease, just as the hours of sunshine varied from week to week, but the final results for the second and third experiments were essentially the same. These facts have a direct bearing on the results obtained in other groups of animals and are of especial interest in connection with effects produced by constant exposure to artificial light.

In attempting to arrive at a conclusion concerning the significance of the results reported, the distinctive features of the reaction displayed by each group of animals should be viewed in relation to the nature of the experimental condition represented. The variable factor in the experiments was the light environment. Other factors were either constant or the variations that occurred were common to the three groups of animals. For reasons that have been explained, the conditions compared were extreme; with one group of animals, light was excluded so that if it were capable of influencing the reaction to infection, this influence would be completely eliminated. The course of disease in these animals was characterized first by uniformity or constancy with respect to both the incidence and the time of occurrence of successive reactions or lesions and second, by a high incidence of generalized syphilis of a severe character. That is, the infection pursued a uniform course from inoculation to the development of outspoken secondary syphilis with comparatively little restraint during the early stages of infection.

A second group of animals was provided with a constant and continuous supply of artificial light of high intensity with a view to affording an opportunity for the development of any effect which the light might be capable of producing, free from any possible disturbing influence of periodic variation. In this case, the severity of the disease diminished with successive experiments as the length of exposure to light increased. There was an increased intensity of primary reactions with a progressive delay and diminished frequency in the occurrence of secondary reactions including metastatic orchitis and generalized

lesions. The picture presented suggested an accentuation of primary reactions with increased effectiveness and consequent delay or prevention of other lesions—a condition almost diametrically opposed to that presented by the animals from which all light was excluded.

It should be borne in mind, however, that placing the animals in darkened or artificially lighted rooms entailed a change in the light environment to which they were accustomed. This may have affected the results of the first experiment in particular and affords a possible explanation for the fact that, among the dark animals, the disease attained its maximum severity in the first experiment, was somewhat milder in the second, and again more severe in the third experiment.

The condition represented by the control animals differed from that of the animals receiving artificial light in three essential respects, namely, the character of the light, and the intensity and constancy of illumination. The results obtained differed also with respect to the general character of the reaction to infection, the relative frequency of occurrence of various reactive phenomena, and especially with respect to the regularity in the evolution of the disease from one experiment to another; but, the final results showed an evident tendency in the same general direction.

On the whole, it may be said that the results tended to conform to the nature of the experimental condition represented. In the control animals, the reaction to infection varied in accordance with the conditions under which the animals lived; the animals provided with artificial light showed a progressive increase in the efficiency of the reaction corresponding with the length of exposure to the light while among those in the dark there was a gradual lowering of the efficiency of the reaction to infection, if we measure efficiency in terms of prevention or delay in the development of successive manifestations of disease.

It is obvious that these effects are attributable to the conditions under which the animals lived rather than to any condition inherent in the material used for inoculation or to chance variation. It is also evident that the course of disease in the three groups of animals may have been influenced in a common direction by a number of unknown factors, including the material inoculated and there is unmistakable evidence of the action of such factors. The differences shown, how-

ever, can be accounted for only on the basis of differences in the light environment, the effect of which may have been accentuated or diminished by the influence of other factors. Moreover, it is apparent that the reaction of animals may be influenced by conditions to which they are exposed either before or after the initiation of the infection.

The mechanism concerned in the production of these effects is unknown, but since it has been shown that the light environment influences the physical constitution of normal rabbits (5) and that the organs most affected are organs that are concerned in the reaction to syphilitic infection (2, 8), it is logical to assume that the effects produced in this case represent expressions of functional activity which are closely related to changes in physical constitution.

Finally, the greater efficiency of the reaction displayed by animals exposed to artificial light as compared with those living in darkness and the maintenance of a better physical condition speak for a beneficial influence of light which is supported by the results obtained in control animals. There are, however, no grounds for assuming that the optimum effect was attained by the particular conditions that were employed in these experiments.

A comparison of effects due to filtered sunlight and to artificial light *per se* cannot be made as the condition complex represented by one form of light was variable and uncontrolled while that of the other was constant. The superiority shown by the artificial light may have been referable to the constancy of exposure rather than to differences in either the quality or the intensity of the light. It is evident that changing conditions of light environment are a disturbing factor and that in extreme cases the occurrence of such changes may completely counterbalance any beneficial effects of the light itself; but, the relative importance of the several factors that determine the effects of light on the animal organism and the general scope of their action are still uncertain.

SUMMARY.

A series of experiments was carried out for the purpose of determining whether the reaction of rabbits inoculated with *Treponema pallidum* might be influenced by their light environment. The condi-

tions compared were (1) diffuse sunlight filtered through window glass and subject to variations due to natural causes, (2) constant and continuous exposure to artificial light with a wave-length of from 3022 to 5790 Ångström units (Cooper Hewitt), and (3) complete exclusion of light.

The results showed clearly that each of these conditions produced a distinctive effect and that the effect tended to conform to the nature of the environmental condition. In general, the efficiency of the reaction to infection increased with the amount of light received and with the constancy of the exposure.

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AN INFECTIOUS GRANULAR VAGINITIS OF COWS.

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PLATE 15.

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An infectious disease of cows characterized by an acute inflammation of the vaginal mucosa and terminating in the formation of raised red nodules studding the mucosa has been recognized in many parts of the world. Hess[1] reported that in certain sections of Switzerland over 60 per cent of the cows suffered with the disease. It is one of the common disorders of cows in this country.

Ostertag,[2] Hecker,[3] and others succeeded in cultivating a Gram-negative streptococcus from the mucopurulent exudate. The organism was described as extracellular and occurred in chains of from 6 to 9. In many instances it was associated with staphylococci and *B. coli*. Ostertag inoculated the vagina of cattle, sheep, goats, pigs, and mares with the streptococcus and reproduced the disease. He points out that Raebiger,[4] Jüterbock,[5] and others reproduced the disease in cows by intravaginal inoculation with a similar streptococcus. Blaha[6] observed in a series of cases bodies embedded in the epithelial cells similar in many respects to those observed in trachoma, which led him to believe that it was a Chlamydozoa infection.

Relatively little concerning the etiology of vaginal infections has been published in this country. Starr[7] noted that the nodules resulted from hyperplasia of the lymph follicles as the result of irritation. He succeeded in cultivating a streptococcus of the *viridans* type from the exudate.

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[1] Hess, cited by Ostertag.[2]

[2] von Ostertag, R., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1913, vi, 269.

[3] Hecker, *Berl. tierärztl. Woch.*, 1900, 445.

[4] Raebiger, W., *Berl. tierärztl. Woch.*, 1907, 254.

[5] Jüterbock, K., *Z. Tiermed.*, 1909, xiii, 354.

[6] Blaha, E. H., *Berl. tierärztl. Woch.*, 1909, xxv, 879.

[7] Starr, L. E., *Vet. Med.*, 1924, xix, 25.

The disease we encountered resembled in many respects the usual granular vaginitis. However, it differed from the latter in that it was often more severe, and streptococci were not found in great numbers. It will be brought out later that the streptococci we isolated are not the microbic incitant of the condition.

History of the Cases.

A considerable proportion of our cases occurred during the months of November and December, 1925, and January and February, 1926. The disease became epidemic during November shortly after a tuberculin test when a large number of newly purchased cows and a considerable number of young native cows were introduced into the herd. In several instances all the newly purchased cows and the young native cows in certain barns were attacked about the same time. The epidemic subsided but sporadic cases continued to appear in native young cows introduced into the herd during the first 5 months of 1926. During this outbreak over 100 cases occurred.

In addition to this material we had access to several cases in cows originating in Ohio and purchased from a dealer, also to cases evidently originating in Oregon among cows shipped from there to New Jersey in special cars. This material convinces us that the infection with which we had to deal is one of considerable distribution in this country.

Characterization of the Disease.

The disease was severe among the newly purchased and young native cows. The vulva was greatly swollen and tender. The visible vaginal mucosa was deeply congested and swollen and the clitoris enlarged and bright red. The mucosa covering the floor and walls of the vagina was sprinkled with numerous, tiny, indistinct, grayish white areas which rapidly coalesced to form large plaques of grayish or yellowish white exudate (Fig. 1). When the exudate was forcibly removed a raw, bleeding, grayish red surface was exposed. Considerable thick mucopurulent exudate often gathered about the clitoris and on the floor of the vagina. The inflammation slowly subsided and the exudate sloughed exposing a granulating surface. The mucosa regenerated but tiny, round, red areas appeared embedded in

the mucous membrane. These enlarged and finally became round, raised red nodules 1 to 2 mm. in diameter. A little mucopurulent exudate frequently persisted about the clitoris for a considerable period.

The lesions in the cows originating in Ohio and Oregon were much less severe. In both groups the vaginal mucosa of a number of animals was sprinkled with the red granules similar to those observed in the severe cases. In other instances a more acute condition was observed, and here the vulva was swollen and tender. The vaginal mucosa was bright red and sprinkled with strings of loosely adherent, yellowish white, purulent exudate. At times small amounts of mucopurulent exudate accumulated on the floor of the vagina. With the subsidence of the acute inflammation the characteristic granules commenced to appear.

As far as we could determine the disease was confined to the vagina. The general health was not noticeably affected. The milk yield remained normal.

Bacteriological Findings.

We attempted to demonstrate the presence of organisms in films of the exudate from fresh cases by means of heat fixation and staining with methylene blue or Gram's method. By such procedures we were able to recognize a relatively few organisms, usually streptococci or micrococci, but in insufficient numbers to account for the lesions. When rapidly dried films were fixed for 3 to 5 minutes in methyl alcohol and then stained for 30 to 40 minutes with a solution consisting of Giemsa's stain 2.0 cc., methyl alcohol 1.5 cc., distilled water 20 cc., or stained with carbolfuchsin diluted 1:20 in distilled water for 1 to 2 hours, we were able to demonstrate a considerable number of tiny delicate rods with well developed polar granules (Fig. 2). In many instances the cytoplasm between the granules stained feebly or not at all, so that the organisms resembled tiny diplococci, shown in Fig. 3.

The exudate is composed largely of leucocytes, epithelial cells, a few endothelial phagocytes, and considerable mucus. It was possible by obtaining portions of the exudate on sterile swabs and bringing the material to the laboratory to cultivate on the ordinary media

certain well defined types of organisms, such as streptococci, staphylococci, *B. coli*, and long, slender, Gram-positive rods, but in no instance was an organism encountered which resembled the bacillus met with in the films. After a considerable number of failures we were successful in obtaining it in pure culture. The procedure finally adopted was to transfer the exudate directly from the cow into the condensation water of a blood agar slant. Agar slants were prepared from veal infusion, and when slanted and cooled, 0.5 cc. of defibrinated horse blood was added. From the first tube, three others were inoculated in series, care being taken to flame the loop between each tube. The tubes were then sealed with sealing wax and incubated for 5 days at 38°C. As a rule Tube 1 contained streptococci and other types of organisms. Tube 2 contained in addition to streptococci clumps of tiny coccoids and a few tiny bacilli with well defined polar granules. Tube 3 was often to outward appearances sterile, or showed an indistinct haze in a narrow zone about the level of the condensation liquid between the tube wall and the agar; examination of the stained films, however, revealed small numbers of tiny coccoids in clumps and occasional well defined rods (Fig. 4). Tube 4 contained a pure culture or remained sterile. Transfers from the tubes containing only the coccoids and rods are usually successful, but the organism is pretty apt to grow only in the condensation fluid or between the agar and glass for four or five generations; after this time delicate flattened colonies with slightly raised centers appear on the slant.

The organism stains poorly after heat fixation, but films fixed in methyl alcohol stain well with Giemsa. It is Gram-negative and non-motile. The morphology varies. A constant finding in cultures is the densely packed masses of tiny coccoids (Figs. 4 and 6) or tiny rods (Figs. 4, 5, 6) containing polar granules. Free forms more or less elongated with well defined granules are likewise present. In later cultures (Fig. 5), the bacilli are larger and stain more deeply. All cultures passed through a phase in which growth was apparently going on more rapidly, but a final adaptation to the medium had not been reached. Here large clumps of the coccoids are plentiful, as well as extremely long filamentous forms containing large masses of deeply stained protoplasm and the tiny granules (Fig. 6). This

phase passes and finally there is a reversion to the clumps of coccoids and the tiny rods with polar granules. Cultures in blood broth reveal the general variations as illustrated in Fig. 7. It is to be observed that considerable variation in size exists. In the films of exudate the bacilli measure from 1 to 2μ in length. Bacilli of this length are common in all the cultures. The coccoids are exceedingly small, 1/3μ, but the probabilities are that they comprise the polar granules of bacilli whose central zones and cell walls fail to stain. The filamentous forms referred to vary from 10 to 45μ in length. Many show a tendency to fragment near the ends. Others may stain irregularly throughout their entire length.

Once a culture is established on blood agar it is readily transferred to blood broth. Thus far it has not been possible to establish growth in coagulated horse serum to which sterile calf serum water has been added or in serum agar. It will grow, however, in the condensation water of plain agar or ascitic fluid containing fresh tissue such as guinea pig spleen or kidney.

In blood broth to which 1 per cent of dextrose, lactose, saccharose, maltose, or mannitol was added, no fermentation was observed after 10 days incubation. Milk heavily inoculated with blood broth culture remained unchanged. Indole was not produced in sugar-free broth containing blood.

It seemed possible from the size of the coccoids that the organism might readily pass through the coarser Berkefeld filters. On four occasions we attempted filtration through candles V and N but the filtrates remained sterile. Inoculations from the filtrates after suitable incubation were also negative.

Pathogenicity of the Bacillus.

Rabbits weighing 2000 gm. withstand 2 cc. of blood broth culture injected intravenously. Guinea pigs of 300 gm. remain well when injected intraperitoneal with 0.5 cc. of culture. 1 cc. may cause death or produce a febrile reaction lasting several days. The injection of 2 cc. has always resulted fatally. Death results from peritonitis in 24 hours. The bacilli are found in the exudate in enormous numbers and can be cultivated from the heart's blood.

Heifer calves, 3 or 4 months old, and 2 year old heifers were in-

oculated into the vagina with culture. In every instance acute inflammation resulted. Granules similar to those observed in the spontaneous disease were always observed after the acute inflammation had subsided. The following experiment affords an example.

The mucosa of the vagina of unbred Heifer 1116 was brushed with a swab immersed in the condensation water of a 3 day blood agar culture of the bacillus in the third culture generation. There was no reaction during the first 24 hours. On the 2nd day the vulva was swollen and tender. The vaginal mucosa was bright red and swollen. Strings of yellowish white, purulent exudate adhered to the mucosa covering the floor and sides. On the 3rd day the swelling was more marked and there was considerable tenderness on manipulation. The whole mucosa was bright red and bled when brushed lightly with a sterile swab. Blood agar inoculated with exudate on this day resulted in pure cultures. The films of the exudate (Fig. 8) showed necrotic epithelial cells, leucocytes, mucus, and a moderate number of the characteristic bacilli. On the 4th, 5th, and 6th days the congestion and swelling were pronounced, and considerable exudate was present about the clitoris and adhered to the walls. Cultures made on the 5th day contained the bacilli. On the 9th day there was more exudate and the whole mucosa appeared to be granulating. After 11 days the mucosa was studded with barely visible, indistinct, grayish white areas. These were a little larger and more red in color on the 12th day, and on the 13th day were recognizable as distinctly visible, raised, red nodules. The nodules increased in size and finally on the 19th day appeared round, sharply raised, firm, 1 to 1.5 mm. in diameter. Cultures made on this day contained the bacilli.

The heifer was slaughtered 89 days after the inoculation. The granules were still visible in the mucosa of the vestibule and walls of the vagina. They did not extend into the uterus. Examination of material fixed in Zenker's fluid and stained with methylene blue revealed that the lymph follicles in the submucosa were hyperplastic. Some follicles were discrete, others were joined by bands of round cells. Over the smaller, more discrete, round celled accumulations the epithelium was normal, but that overlying the larger follicles was heavily invaded with round cells (Fig. 9).

Mention has been made that many of the cultures from the spontaneous cases contained streptococci. These were all of the non-hemolytic or green-producing type and resembled those described by F. S. Jones[8] as the type usually found in the vagina of healthy cows. We inoculated four heifer calves with the cultures. No inflammation resulted, but on subsequent inoculation with pure cultures of

[8] Jones, F. S., *J. Exp. Med.*, 1918, xxviii, 735.

the rods from vaginitis acute inflammation resulted followed by the formation of typical granules. In these experiments the granules appeared from 5 to 7 days after infection.

We were unable to obtain material for histological study from acute spontaneous cases. On several occasions old cows leaving the herd for various reasons were inoculated into the vagina with material from severe cases, but the animals failed to contract the disease. They had probably passed through an attack of the disease and were resistant. Calves as a rule respond moderately only to inoculation with infectious material. In certain instances such inoculations induced severer inflammation and afforded some insight into the nature of the acute process.

The mucosa of the vagina of Calf 1240, 4 weeks old, was brushed with a swab containing exudate from two spontaneous cases. The usual type of acute inflammation followed. 4 days later the calf was slaughtered. At antemortem examination the mucosa was scarlet and sprinkled with strings of tenacious, yellowish white exudate. Evidently the method of slaughter, similar to that used in abattoirs, caused a blanching of the vaginal mucosa, since it was of a pale yellowish pink color along the floor and walls. Anterior to the clitoris was a red area situated within the mucosa. Other portions of the mucosa contained a few tiny, sunken, irregular, red patches. A little mucopurulent exudate was present on the mucosa of the floor. Inoculation of blood agar with this material developed cultures of the characteristic bacilli. The uterus was normal.

Histological examination of fixed and stained material revealed well defined necrosis of portions of the epithelium. In portions most of it had apparently sloughed so that the surface was covered with a thin layer of necrotic epithelial cells, degenerated leucocytes, and a little fibrin. The submucosa was edematous and infiltrated with leucocytes and round cells. The blood vessels were moderately engorged with red cells and contained excessive numbers of leucocytes and round cells. Other portions of the epithelium were intact. Lesions were not found in sections of the uterus.

From clinical examination of cases, exudate from such cases, and the histological material, we feel that the process may in part be pieced together. The bacilli attack the mucosa in certain foci. Here necrosis of the epithelium results. A little fibrin may exude beneath the epithelium. Leucocytes in large numbers invade the mucosa. The submucosa is invaded by round cells and leucocytes. The exudate and mucosa slough, followed by regeneration accompanied by

large accumulations of round cells in follicle-like masses in the submucosa. The large amount of exudate in the outbreak may be explained by a heavy infection with the bacilli so that the necrotic areas occurred close together and gave the appearance of a continuous membrane.

DISCUSSION.

It is apparent that in the large outbreak we had to deal with a severe type of inflammation of the vagina. In certain respects the type of disease differed from that usually considered typical of granular vaginitis and that encountered in our cases drawn from other sources. From each group, however, we succeeded in isolating a similar organism. The acute inflammation in all cases terminated in the appearance of the characteristic granules in the submucosa.

In the outbreak several factors contributed to exalt the severity of the infection. The disease could easily be spread by thermometers during a tuberculin test. There had been a large number of young cows recently introduced into the herd and these animals with young native cows represented a large number of susceptible individuals. The method of spread was apparently direct from cow to cow, since in this herd animals are brushed and curried before milking. It appeared that all cows on one side of a barn were infected at about the same time. A favorable opportunity was thus created for the rapid spread of the inciting organism to a large number of relatively highly susceptible cows.

With the culture isolated from the severe cases it was not possible to produce the severe type of disease. We simply reproduced a condition similar to that found in the cows from Oregon and Ohio. It must be remembered, however, that we used for the purposes of inoculation relatively small doses of a feebly growing culture. The experimental disease was always well pronounced, accompanied by a mucopurulent exudate, and the acute process terminated in the formation of the characteristic granules. In sharp contrast are the entirely negative results after inoculation with non-hemolytic streptococci also isolated from the vaginal exudate.

Although the question of immunity produced by an attack is not

definitely proved, yet considerable resistance seems to result. We have noted on several occasions that old cows standing between severe cases did not contract the disease although infectious material must have been frequently brushed into the vagina. In two instances cows which had been exposed during the outbreak were inoculated intravaginal with material from severe cases and failed to develop the disease.

The bacillus isolated is apparently one not described before. That its distribution is widespread is indicated by its presence in cows from Oregon, Ohio, and New Jersey. Morphologically it resembles in certain respects the bipolar group in having polar granules and giving rise to the long involution forms during certain phases. However, it differs in many respects from usual organisms of this type. It fails to ferment dextrose, or any of the carbohydrates, produces no indole, and possesses relatively no pathogenicity for rabbits. It grows only in media containing blood or bits of tissue, and then only in the parts of the tubes containing little free oxygen. It is not an anaerobe. Thus far it has failed to grow in the unsealed tube. It possesses no hemolytic or proteolytic properties. At present its identification rests largely on morphological criteria, the difficulty with which it stains, and the inability to grow in media which do not contain fresh blood or tissue.

SUMMARY.

A disease of cows characterized by swelling of the vulva, acute inflammation of the vaginal mucosa, accompanied by a more or less profuse mucopurulent exudate is described. After the acute inflammation subsides the mucosa becomes studded with tiny, round, raised, red nodules which persist 2 or 3 months or longer. The acute lesion consists in necrosis of the epithelium and accumulations of leucocytes and round cells in the edematous submucosa. The nodules in the later stages are made up of densely packed masses of lymphocytes in the submucosa which force the epithelial layer outward.

A Gram-negative bacillus with tiny polar granules was found in the exudate. It measures 1 to 2μ in length and stains with difficulty. The organism was obtained in pure culture by inoculating the exudate

into tubes of slanted agar to which defibrinated horse blood had been added. Growth occurs only in sealed tubes. The organism possesses slight pathogenicity for guinea pigs. When freshly isolated cultures were introduced into the vagina of heifers or young calves, acute inflammation resulted which terminated in the characteristic granular stage of the disease.

EXPLANATION OF PLATE 15.

FIG. 1. Natural infection. Severe inflammation of the vagina. Note patches of exudate on the mucosa. About 1/5 natural size.

FIG. 2. The bacilli along the border of heavily stained mass of exudate. Spontaneous case. Methyl alcohol fixation. Dilute carbolfuchsin stain. × 1000.

FIG. 3. Exudate from spontaneous case, showing two large leucocytes and two bacilli with polar granules. Methyl alcohol fixation. Giemsa stain. × 1000.

FIG. 4. The condensation fluid from an original blood agar culture 5 days old. Note the clump of coccoids, a single well stained bacillus, and an elongated form. A red blood cell is also present. Giemsa stain, after methyl alcohol fixation. × 1000.

FIG. 5. The same culture as Fig. 4, in the fourth generation. Condensation fluid of a 5 day blood agar culture. The bacilli are larger and stain more intensely. A red blood cell is included in the field. Giemsa stain. × 1000.

FIG. 6. The same culture as Figs. 4 and 5, in the fourteenth culture generation. Condensation fluid of a 3 day blood agar culture. Three forms are illustrated, a large clump of short coccoids, a few individual bipolar forms, and two long filaments, one of which shows a tendency to fragment. Giemsa stain. × 1000.

FIG. 7. A blood broth culture, in the third generation, 3 days old. Giemsa stain. × 1000.

FIG. 8. The bacilli in the vaginal exudate from Heifer 1116, 3 days after intravaginal inoculation with culture. Giemsa stain. × 1000.

FIG. 9. Section of the mucosa of the vagina of Heifer 1116, 89 days after inoculation with culture. Note the infiltration of round cells in the mucosa and the dense accumulation of round cells in the submucosa. Zenker's fixation. Eosin-methylene blue stain. × 66.

(Jones and Little: Infectious vaginitis of cows.)

EPIDEMIOLOGICAL STUDIES ON RESPIRATORY INFECTIONS OF THE RABBIT.

IX. The Spread of Bacterium lepisepticum Infection at a Rabbit Farm in New City, N. Y.

An Epidemiological Study.

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A comprehensive experimental study of the mode of spread of a bacterial disease demands knowledge of its natural epidemiology (1, *a*). This includes data describing the natural prevalence and distribution of the inciting organism, the frequency of various bacterial types in nature, their normal portal of entry into the body of the host, the different responses of the host to the microbe, and the frequency of each response under varying hereditary and environmental conditions. Such information regarding the natural prevalence of an animal disease has at least two important uses in experimental epidemiology,—first, it enables one to induce the infection experimentally, under conditions which simulate nature closely, thereby reducing the number of disturbing artifacts; second, it serves as a control with which to evaluate the conclusions derived from the precise, yet necessarily limited experiments of the laboratory.

As an aid to our experimental studies of *Bact. lepisepticum* infection of rabbits, we have observed the native disease as it occurs among the stock at The Rockefeller Institute and at a rabbit farm at New City, N. Y. The findings in the former group, during the years 1923 and 1924, may be reviewed briefly as follows.

Bact. lepisepticum infection has been found present in about 60 per cent of the rabbits at The Rockefeller Institute (1, *b*). From 20 to 40 per cent of the unused, "normal" stock showed clinical or pathological evidence of snuffles, 5 per cent

evidence of otitis media (1, *c*), 30 per cent were "healthy," nasal carriers (1, *d*), and 20 per cent appeared normal and were not carriers. Among the animals subjected to operation, or otherwise used for experimental purposes, the percentage of infection was greater—in one such series, 40 per cent showed evidence of snuffles as compared to 20 per cent in the corresponding control group (1, *e*); in another, otitis media, 45 per cent, as compared to 5 per cent in unused stock (1, *c*). Inoculations of injurious substances were found to increase the incidence of the "spontaneous" infection by as much as 50 per cent (1, *e*). Both "D" and mucoid types of *Bact. lepisepticum* were encountered at this time (1, *f*).

The New City group of rabbits was studied in a somewhat similar manner and the findings are described in the present paper.*

Source of Material.

The present studies were made at a commercial rabbitry in New City, N. Y.[1] The farm is situated in open country, 200 feet above sea level, in a sparsely populated community. The buildings are placed on a tract of land comprising several acres and are isolated by means of a surrounding wire fence, 8 feet high. A number of watch dogs serve as further protection from animal marauders. Three buildings were in use during the first months of our observations; later, a fourth was added. In general architecture, they are all similar (Text-fig. 1). They measure 100 feet by 20 feet, with the long dimensions facing east and west. Each building contains two rows of wooden cages, running the length of the structure, arranged in tiers of four from ceiling to floor. About 368 cages, measuring 5 feet by 2.1 feet by 1.6 feet each, are contained in each building. The cages consist of a front runway facing toward the open east or west exposure, and a rear partition, kept relatively dark and used for breeding. Each house is cared for by two men who maintain a uniform and efficient system of cage cleaning and feeding throughout. Temperature fluctuates during the year according to outside weather conditions. No artificial heat is used. The side doors, east and west, are thrown open whenever possible, thus making conditions practically "out-of-doors." Food consists of hay, a grain mixture of oats, oatmeal, and wheat, fresh carrots, cabbage, and greens, according to the season. During the winter cold storage carrots are used.

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* Since submitting the manuscript for publication data through October have been obtained and the text-figures and text have been changed accordingly.

[1] We wish to thank the owners of this rabbit farm for permitting us to make this investigation. Especially to Mr. Karl Haack we express our appreciation. He showed at all times interest and cooperation, placing the farm entirely at our disposal, giving us assistance in the examinations, special trucks for conveying animals to the laboratory, and many other facilities, without which this work would have been impossible. To him, therefore, is due much of the credit for the progress of the investigation.

The rabbit population is composed almost entirely of pedigreed and registered stock. About 50 per cent are Chinchillas, 15 per cent Blue Beverens, 10 per cent Havanas, 10 per cent Belgian hare crosses with Chinchillas, and 15 per cent Gaudas, Lilacs, and Angoras. Pure line breeds were thus available for comparative study.

The animals are distributed as follows: Building 2, all breeds; No. 3, Chinchillas; No. 4, Chinchillas, Blue Beverens, and Angoras; No. 6, Chinchillas. Each rabbit 6 months old or over occupies a separate cage. The females are bred four or five times a year; four males are used for twenty females. Complete breeding records are kept. The young remain in the cage with the mother until they are old enough to be placed in individual cages.

TEXT-FIG. 1. Building 6, showing open side doors and arrangement of individual rabbit pens. Photograph by Edwin Levick furnished by Mr. Haack.

Methods of Study.

The spread of *Bact. lepisepticum* infection among this group of rabbits was studied as follows. Animals 6 months of age or over, occupying individual cages, were selected for observation. The number of these varied from 841 to 1325. The young were disregarded.

(1) From November, 1924, until November, 1926, complete mortality records were kept. (2) From November, 1924, until April, 1925, every rabbit that died was wrapped in paper, placed on ice, and sent to The Rockefeller Institute, where a complete autopsy was performed and cultures were taken. (3) During November, 1924, February, May, and August, 1925, a clinical examination of each rabbit was made. Its general condition was noted, signs of *Bact. lepisepticum* infection were searched for carefully, and other abnormalities were recorded. (4) Finally, a group of clinically normal Chinchillas and Blue Beverens was tested for fre-

TABLE I.

Summary of Data on the Mortality and Incidence of Snuffles among the Rabbits at the New City Farm, N. Y.

Date of observation	Total No. of rabbits examined	Total per cent snuffles	Total mortality per 1000	*Bact. lepisepticum* infection mortality per 1000	Intestinal infection mortality per 1000	Miscellaneous causes mortality per 1000	Comparative snuffles and mortality rates in different breeds of rabbits													
							Chinchilla breed					Havana breed				Blue Beveren breed				
								Mortality		Snuffles			Mortality		Snuffles			Mortality		Snuffles	
							Total No.	No.	Per cent	No.	Per cent	Total No.	No.	Per cent	No.	Per cent	Total No.	No.	Per cent	No.	Per cent
1924																					
November	1016	46.2	30.5	14.75	8.86	6.9	576	19	3.3	285	49.4	117	5	4.2	50	42.7	82	1	1.2	20	24.3
December	—	—	*48.3	23.6	16.75	7.9	—	27	4.7	—	—	—	8	6.8	—	—	—	4	4.8	—	—
1925																					
January	—	—	*40.3	24.6	12.8	2.95	—	31	5.3	—	—	—	9	7.7	—	—	—	1	1.2	—	—
February	1015	48.9	27.6	12.8	10.9	3.94	503	19	3.8	268	53.2	118	4	3.4	64	54.2	112	1	0.9	39	34.8
March	—	—	*47.4	27.6	11.8	7.9	—	26	5.1	—	—	—	7	5.9	—	—	—	2	1.8	—	—
April	—	—	*75.9	—	—	—	—	40	7.9	—	—	—	15	12.7	—	—	—	3	2.6	—	—
May	1116	49.1	106.7	—	—	—	528	64	12.1	287	54.3	118	18	15.3	68	57.6	196	11	5.6	54	27.5
June	—	—	*89.5	—	—	—	—	57	10.8	—	—	—	11	9.3	—	—	—	4	2.0	—	—
July	—	—	*28.7	—	—	—	—	20	3.8	—	—	—	4	3.4	—	—	—	1	0.5	—	—
August	1308	43.3	28.1	—	—	—	564	23	4.0	284	50.3	117	6	5.2	78	66.6	214	1	0.3	68	24.1
September	—	—	*31.2	—	—	—	—	17	3.0	—	—	—	4	3.4	—	—	325	8	2.5	—	—
October	1325	—	28.7	—	—	—	630	17	2.7	—	—	—	4	3.4	—	—	350	5	1.4	—	—
November	1310	—	24.4	—	—	—	620	10	1.6	—	—						350	6	1.7	—	—
December	1113	—	39.5	—	—	—	550	18	3.3	—	—						300	14	4.7	—	—
1926																					
January	1087	—	37.8	—	—	—	550	22	4.0	—	—						285	9	3.20	—	—
February	971	—	36.0	—	—	—	490	16	3.3	—	—						260	5	1.9	—	—

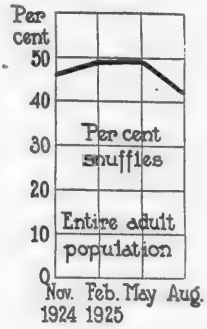
March	954	—	24.0	—	—	—	490	14	2.9	—	—					230	4	1.7	—	—
April	1016	—	33.5	—	—	—	490	22	4.5	—	—					250	6	2.4	—	—
May	961	—	34.0	—	—	—	441	26	5.9	—	—					240	4	1.7	—	—
June	981	—	26.0	—	—	—	458	19	4.1	—	—					237	6	2.5	—	—
July	868	—	50.0	—	—	—	430	22	5.1	—	—					206	13	6.3	—	—
August	841	—	19.0	—	—	—	470	7	1.5	—	—					185	3	1.6	—	—
September	893	—	25.0	—	—	—	489	13	2.7	—	—					220	0	0	—	—
October	855	—	14.0	—	—	—	450	6	1.3	—	—					222	0	0	—	—

* Rates based on total population of last recorded census.

quency of nasal carriers of *Bact. lepisepticum.* Thus, we have available for comparative study mortality, morbidity, and carrier records on several pure line strains of rabbits over a considerable period of time.

RESULTS.

The results of these observations are summarized in Tables I to III, and are described under three main heads,—those pertaining to (1) the entire rabbit population; (2) to various breeds; and (3) to individual animals. We have included only such data as seemed significant; a mass of incidental information collected for the sake of completeness will be referred to briefly.



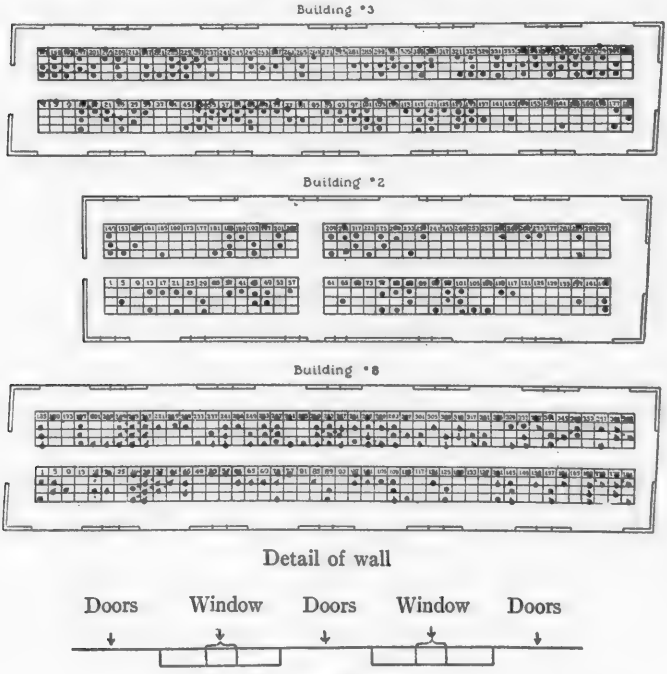
TEXT-FIG. 2. Incidence of clinical snuffles among the adult rabbits at New City farm, N. Y.

Bacteriology.

At the outset, it may be stated that with very few exceptions *Bact. lepisepticum* was obtained from the cases of pneumonia and septicemia examined. It was always recovered from the nasal passages of animals showing clinical evidence of snuffles. Furthermore, a considerable percentage of apparently healthy animals proved to be nasal carriers of this organism.

In every instance the bacterial type obtained was mucoid, with cultural and serological characteristics similar to mucoid strains obtained from rabbits at The Rockefeller Institute and from a rabbit colony at Ray Brook, N. Y. (2). The virulence was uniform and constant; instilled intranasally into young animals, pneumonia was

infrequently induced, snuffles in 40 to 50 per cent of the animals, and "healthy" nasal carriers in 20 to 30 per cent. Detailed studies of some of the strains are described elsewhere (1, *f*).

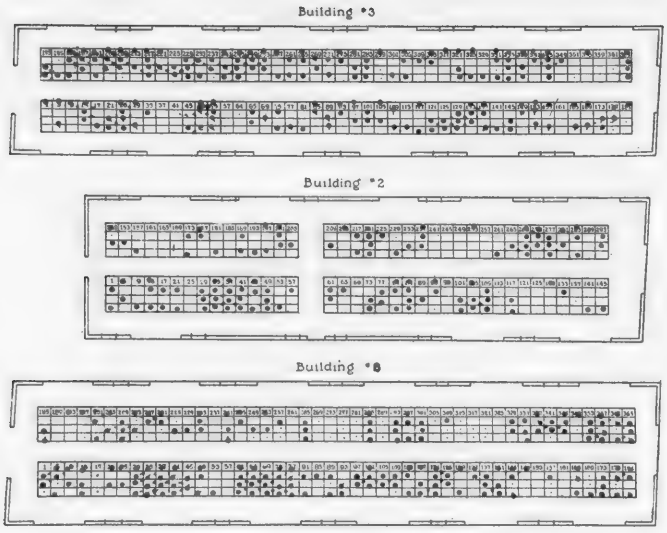


TEXT-FIG. 3.

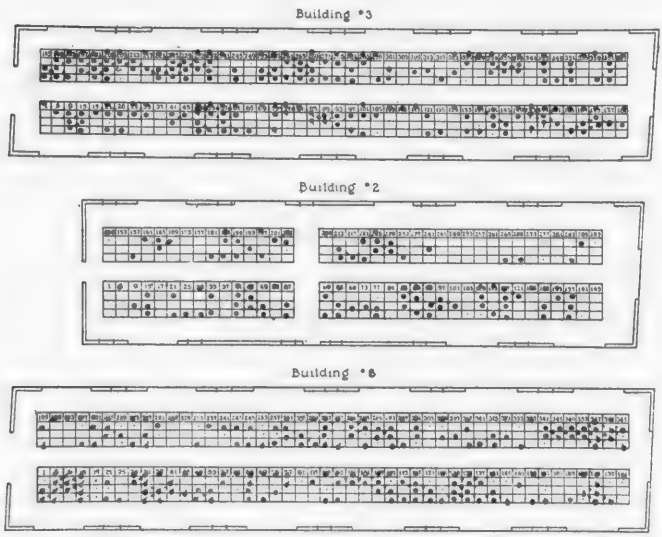
TEXT-FIGS. 3 to 6. Distribution of clinical snuffles among the adult rabbits at New City farm, N. Y., November, 1924 (Text-fig. 3), February, 1925 (Text-fig. 4), May, 1925 (Text-fig. 5), and August, 1925 (Text-fig. 6).

Findings Referable to the Total Adult Population.

The incidence of clinical snuffles throughout the total adult population was determined in November, 1924, and in February, May, and August, 1925 (Table I). Cases were found to differ in severity and to vary in this respect from month to month. However, all showed the white, purulent, nasal discharge, matted paws, heavy breathing, and sneezing characteristic of *Bact. lepisepticum* infection. Indeed,



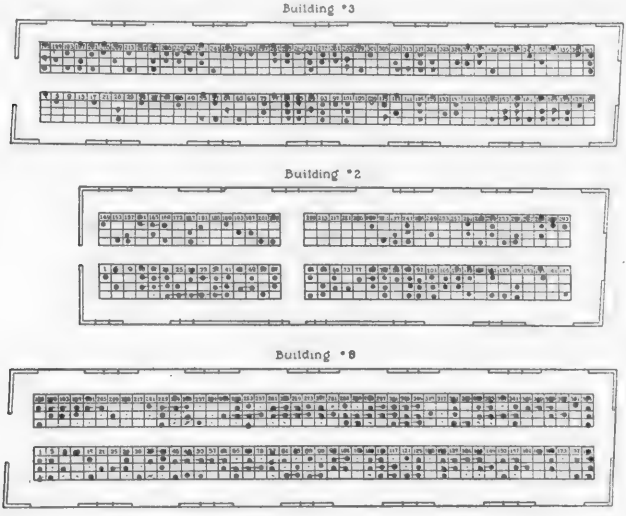
TEXT-FIG. 4. Clinical snuffles, February, 1925.



TEXT-FIG. 5. Clinical snuffles, May, 1925.

whenever cultures were taken from the nasal passages, *Bact. lepisepticum* of the mucoid type (1, *f*) was obtained in great numbers.

The results of these examinations are plotted in Text-fig. 2. The rate per hundred of clinical snuffles rose from 46 in November to 49 in February and May, and dropped to 42 in August. Thus a definite spring rise was noticeable, similar to that observed among the rabbits at The Rockefeller Institute. Among this latter group the average percentage of snuffles varied between about 20 and 60 per cent and exhibited definite spring and fall rises (1, *b*).



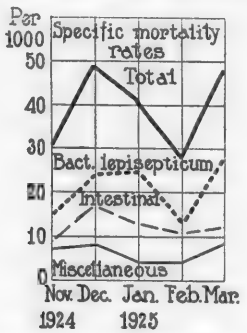
TEXT-FIG. 6. Clinical snuffles, August, 1925.

The distribution of the snuffles cases is charted in Text-figs. 3 to 6. In Text-fig. 3, the spot map for November, 1924, the infection is shown to be present generally throughout the buildings but tending to a focal distribution within each. Thus, in Building 3, definite areas of snuffles are indicated from Cages 13 to 33, 49 to 77, 89 to 109, and 117 to 137. On the opposite tier, there appear to be more cases at either end than in the middle. In Building 2, the infection was less frequent and more scattered. Text-figs. 4 to 6,

which indicate the February, May, and August distribution, show the various foci spreading locally from cage to cage, or disappearing in the same manner.

These maps, together with the mortality records, were useful in demonstrating two facts; first, that *Bact. lepisepitcum* infection in the various buildings was widespread and relatively similar in amount, and second, that the location of doors and windows bore no special relation to the extent or distribution of the disease.

Specific mortality rates from November, 1924, to April, 1925, were obtained by autopsying each rabbit dying within this period and



TEXT-FIG. 7. Specific mortality rates among the adult rabbits at New City farm, N. Y.

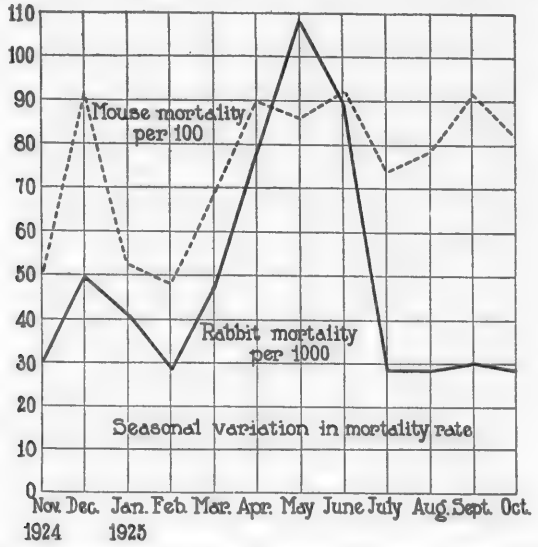
determining as far as possible the cause of its death (Table I). *Bact. lepisepticum* pneumonia and septicemia proved responsible for more than 50 per cent of the total mortality, intestinal disturbances of undetermined etiology[2] for about 30 per cent, and miscellaneous causes for less than 20 per cent (Text-fig. 7).

Text-fig. 7 shows that the fluctuations in deaths due to *Bact. lepisepticum* coincided with the variations in total mortality. During March, 1925, modifications in the green food diet were instituted and thereafter it became apparent, although no autopsies were performed,

[2] No recognized pathogenic organism was recovered from these cases. Occasionally, pin-worms were present, but not in sufficient numbers to be considered the cause of death. Distention of the ileum, cecum, and colon, with occasional subserous petechiæ and flecks of fibrin were the only pathological findings at autopsy.

that deaths from intestinal disturbances were reduced and that at least 75 per cent of the total mortality was the result of *Bact. lepisepticum* infection. Hence, it will be assumed that the trend of the total mortality curve throughout the 2 year period of observation is a general index of mortality fluctuations due to *Bact. lepisepticum.*

The rate of total mortality per month from November, 1924, to November, 1926, is shown in Text-figs. 8 and 9. During both the 1924–25 period (Text-fig. 8) and the 1925–26 period (Text-fig. 9),



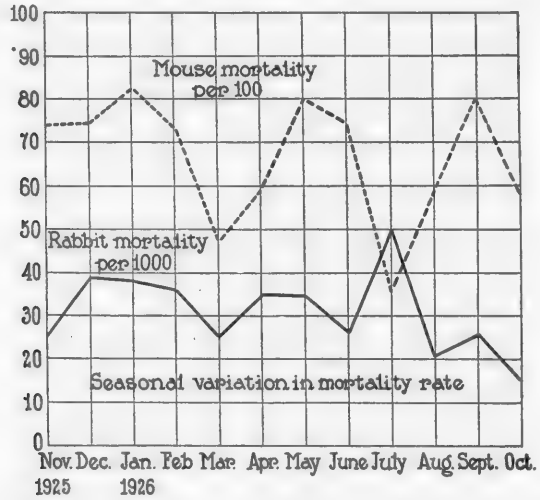
TEXT-FIG. 8. Fluctuations in total mortality of adult rabbits at New City farm, N. Y., 1924–25.

fluctuations occurred in well defined waves, being highest in the spring, low in summer, with a rise again in the fall.

A second curve is included on each chart, representing monthly variations in mortality of mice following the administration of a fixed dose of mouse typhoid bacilli.

The experiments with mice were carried out in such a way as to eliminate as far as possible all disturbing variables (1, *a*). On about the 15th of each month, 50 mice of the inbred Rockefeller Institute strain, 12 to 14 weeks old, weighing

18 to 20 gm. each, bred and raised under the same environmental conditions, given the same diet since birth, and free of any previous exposure to these organisms, were given *per os*, by stomach tube, a fixed dose of about 4,000,000 mouse typhoid bacilli from a standard fluid culture obtained from an agar slant kept at 4°C. (3). Because of the control and elimination of variables in these experiments and because the mortality of a number of groups of similar mice inoculated at the same time differs by a daily average of less than 5 per cent, we consider the wave-like fluctuations in mouse typhoid mortality to be due to variables associated with the season of year.

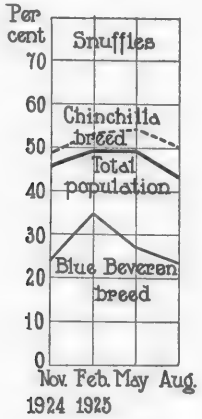


TEXT-FIG. 9. Fluctuations in total mortality of adult rabbits at New City farm, N. Y., 1925–26. From November, 1925, to April, 1926, the mouse curve appears as a fine dotted line. This has been done to indicate a slight change in the technique of the mouse experiments during this period, which changed the absolute values but not the trend.

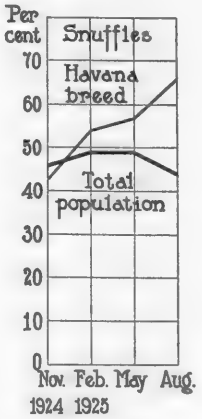
For 2 years the fluctuations of the mouse typhoid and rabbit mortality curves have shown a remarkable degree of similarity. During 1924–25 (Text-fig. 8) the peaks in December, May, and September, and low points in February and July coincided, while in 1925–26 (Text-fig. 9) the peaks in January, May, and September, and the depressions in November and March were again quite similar. We regard the mouse typhoid curves as quantitative measurements

of the influence of seasonal variables on mortality from mouse typhoid under the conditions of this experiment, and in calling attention to the striking similarity of these curves to the total rabbit mortality in the community at New City,[3] we suggest the possibility that the latter, although relatively uncontrolled, are nevertheless to be interpreted as expressions of variations in host resistance associated with the seasons.

These several observations relative to the total rabbit population may be summarized briefly as follows. The incidence of clinical



TEXT-FIG. 10.



TEXT-FIG. 11.

TEXT-FIG. 10. Comparative incidence of snuffles of Chinchilla and Blue Beveren breeds.

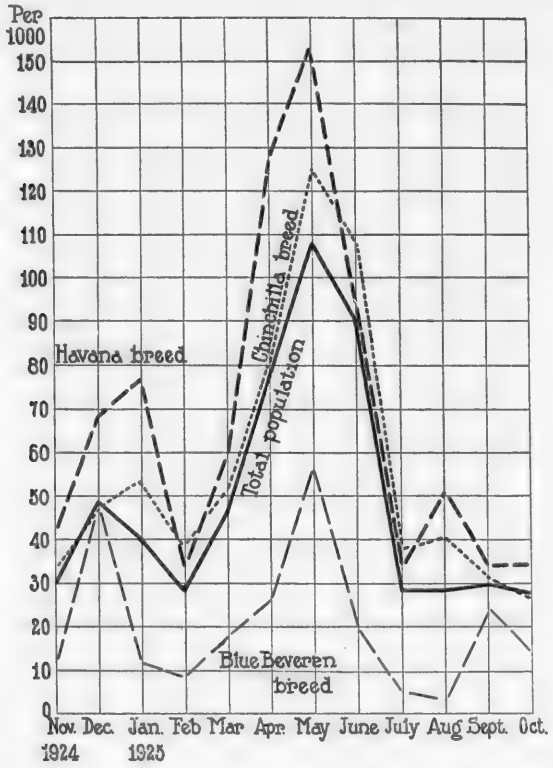
TEXT-FIG. 11. Incidence of snuffles among the Havana breed.

snuffles in the group of about 1000 adult rabbits varied from 46 to 49 to 42 per cent during a period of 10 months, and showed a definite spring rise. The total mortality among these animals, 50 to 75 per cent of which proved to be due to *Bact. lepisepticum* infection, showed wave-like fluctuations over a period of 2 years. High peaks occurred in the spring and fall, and low points were reached in the summer.

[3] The mortality rates of the industrial policy holders of the Metropolitan Life Insurance Company fluctuate in a manner quite similar to the curves in Text-figs. 8 and 9. In them, however, the high spring peak appears in March and April.

Findings Referable to Different Breeds of Rabbits.

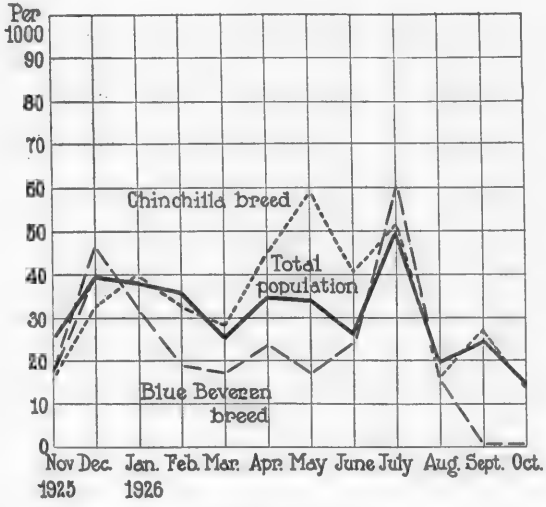
The fact that the rabbits composing this population were originally all pedigreed stock of several definite and well established breeds, and that they have continued to be carefully inbred, afforded a remarkable



TEXT-FIG. 12. Comparative mortality rates of Chinchilla, Havana, and Blue Beveren breeds, 1924–25.

opportunity to compare the responses of these different animal breeds to a native disease. *Bact. lepisepticum* infection was widespread throughout the four buildings. Probably, therefore, all breeds were continually exposed to a relatively similar dose of organisms. Likewise, other factors inherent in food and environment which might

affect the behavior of individuals or groups toward a harmful agent were relatively uniform. Hence it appeared not unlikely that under these conditions any differences in the response of one or another breed of rabbits to *Bact. lepisepticum* infection could be attributed to factors inherent in that special breed. Text-figs. 10 to 13 and Tables I to III strongly favor this probability in showing that the various breeds do in fact exhibit consistent and significant differences in mortality, occurrence of snuffles, and *Bact. lepisepticum* carrier rates.



TEXT-FIG. 13. Comparative mortality rates of Chinchilla, Havana, and Blue Beveren breeds, 1925–26.

95 Chinchilla Giants, 158 Belgian hare-Chinchilla crosses, 37 Gaudas, 25 Blue Gaudas, and 14 Blue Flemish animals were studied, but their numbers seemed too small to include in the charts and tables. It is sufficient to state that the snuffles incidence and mortality per cent of the Chinchilla Giants was consistently greater than that of the Chinchillas, that the Belgian hare-Chincihilla crosses were affected to the same degree, and the remainder to a less degree than the Chinchillas.

In Text-fig. 10, the per cent *incidence of clinical snuffles* among about 550 Chinchillas is compared with that of from 82 to 214 Blue Beverens and also with the entire population. Both breeds showed a fluctuating rate which was highest in the spring. Throughout the 10 months period, the per cent of infected Chinchillas was nearly twice that of the Blue Beverens.

Text-fig. 11 shows the incidence of snuffles among 117 Havanas. In this case, the rate rose steadily from the general average to a point higher than that for any other breed.

Total mortality per 1000 among these breeds was observed from November, 1924, to November, 1926. At least 75 per cent was due to *Bact. lepisepticum* infection, hence there are available for comparison 2 years' records of morbidity (Text-figs. 10 and 11) and mortality (Text-figs. 12 and 13) from *Bact. lepisepticum* infection. The curves in Text-figs. 12 and 13, which describe the total mortality of the various breeds, bring out two facts clearly. First, that the seasonal fluctuations in mortality of the entire population are reflected in each separate breed—Chinchillas, Havanas, and Blue Beverens show a spring and fall rise and a summer drop in mortality similar to those of the total rabbit population and the experimental mouse typhoid series. Second, that the mortality among the Blue Beverens was consistently lower than that of the Chinchillas or Havanas. Only once during the 1924–25 period (Text-fig. 12), and three times during 1925–26 (Text-fig. 13) did the death rate of the former equal or exceed that of the latter.[4]

Finally, *the carrier rate* among clinically healthy Chinchillas was compared with that among similar Blue Beverens. The determinations were made as follows.

15 cm. Petri dishes containing 0.5 per cent blood agar were carefully packed and taken by automobile to the New City farm. Sterile swabs were passed into the nares of selected animals and then streaked over the agar surface of the plates. The cultures were then brought back to The Rockefeller Institute, incubated, and studied in the usual manner (1, *b*, *d*). Only rabbits free of nasal discharge and matting of the fur on the fore paws were tested. In all, 142 Chinchillas and 76 Blue Beverens were examined. Table II shows the results.

[4] The Havana breed, which suffered the greatest mortality, was so reduced in numbers that their record was discontinued in Text-fig. 13.

42 per cent of the Chinchillas and 26 per cent of the Blue Beverens proved to be carriers of *Bact. lepisepticum* on the first examination. A second and third swabbing of the negative Chinchillas increased their carrier rate to 47 per cent and at the same time indicated the usual 5 to 10 per cent error in the results of a single test (1, *b*, *d*). The relative difference between the two carrier rates—Chinchillas 42 per cent, Blue Beverens 26 per cent—is, however, considerable and entirely consistent with the differences between their morbidity and mortality rates.

In brief, these several observations have shown that the rates of snuffles morbidity and mortality among the special breeds of rabbits at the New City farm display the same seasonal fluctuations as do those of the entire population, and that in regard to incidence of

TABLE II.

Carrier Rate of Bact. lepisepticum among the Chinchilla and Blue Beveren Breeds at the New City Rabbit Farm.

Date	Breed	No. of test	No. of animals tested	Carriers of *Bact. lepisepticum*	
				No.	Per cent
April 18–22, 1925....	Chinchilla	1	142	60	42
"	"	3		67	47
May 7–9, 1925.....	Blue Beveren	1	76	20	26

Bact. lepisepticum carriers, snuffles cases, and mortality, these breeds show definite and consistent differences.

Findings Referable to Different Individual Animals.

For a period of 10 months, records were kept on each individual adult rabbit at the farm. In November, 1924, February, May, and August, 1925, the general condition of each and the presence or absence of snuffles were noted. During this time many animals died, some of which were autopsied, and a number of replacements were made. All these data are of interest but cannot be included in the present communication. We have therefore summarized the records of 828 animals which had been observed from 7 to 10 months and

have grouped them by means of an arbitrary classification into a table (Table III).

The figures were obtained as follows. Only those animals observed from 7 to 10 months were included under "Total number in selected group." In the third column, the entire mortality among these animals during the 10 months period is recorded. Then from a tabulation of the records of the survivors, the cases were classified as follows, in the order of severity of clinical reaction to *Bact. lepisepticum:* (1) clinical snuffles, 10 months; (2) clinical snuffles at the outset, disappearing and reappearing; (3) clinically normal at first, later developing well marked and persistent snuffles; (4) clinical snuffles at the outset, later disappearing; (5) clinically normal at the outset, developing snuffles which later disappeared; and finally, (6) clinically normal cases throughout the 10 months period. The occasional otitis media, pneumonia, torticollis, ear canker, diarrhea, etc., cases are omitted from this table because they total less than 1 per cent.

TABLE III.

Reactions of Selected Groups of Rabbits to Bact. lepisepticum.

Breed	Total No. in selected group	Mortality (10 mos.)		Clinical snuffles 10 mos.		Clinical snuffles 10 mos., with remissions		Clinically normal at outset, later developing snuffles		Clinical snuffles at outset, later disappearing		Clinically normal at outset, developing snuffles, later disappearing		Clinically normal, 10 mos.	
		No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Chinchillas.....	578	286	49.5	59	10.2	13	1.8	88	15.2	40	6.9	25	4.3	67	11.6
Havanas.....	144	86	60.0	14	9.7	0	0.0	26	18.5	1	0.7	3	2.1	14	9.8
Blue Beverens....	105	25	23.8	6	5.7	2	1.9	12	11.4	6	5.7	12	11.4	42	40.0

The Havana group showed the most marked reaction to the presence of *Bact. lepisepticum.* 60.0 per cent died, 28.2 per cent had, or developed severe snuffles, and only 9.8 per cent remained clinically normal. Among the Chinchillas as well the effect was severe. 49.5 per cent died, 27.2 per cent showed snuffles during the entire period, or developed it early, and 11.6 per cent appeared clinically normal. The Blue Beverens suffered the least, 40.0 per cent remaining entirely healthy; 19.0 per cent were infected with snuffles at the outset or early during the period, and 23.8 per cent died during the 10 months.

The point deserving special mention, however, is the marked difference in reaction of the individual animals to the presence of *Bact. lepisepticum*. Within each breed there were rabbits which showed no evidence of the disease and others which succumbed; while between these two extremes every gradation was encountered. These differences cannot possibly be due to chance, for it is improbable that the same 67 Chinchillas, the same 14 Havanas, and the same 42 Blue Beverens should have appeared normal on at least four different occasions, over a period of 10 months. Furthermore, the differences in behavior arose under apparently similar environmental conditions and in communities where the bacteria were so widespread that dosage must be considered more or less the same for the entire population. It seems reasonable, therefore, to attribute these variations in animal response, like others which we have noted (1, *g*), to variables inherent in each individual concerned.

DISCUSSION.

The observations on "spontaneous" *Bact. lepisepticum* infection among rabbits have been made under peculiarly advantageous circumstances. Thoroughbred animals, raised in a uniform environment and exposed more or less equally to the risk of infection, comprised the population. Records have been kept of large numbers of individuals over a considerable period of time. The data are therefore relatively free from complicating variables and may be used to advantage in planning and testing the significance of experimental studies.

Investigations dealing with seasonal influences and differences in racial, familial, and individual constitutions show an increasing tendency toward control of complicating variables. Under such circumstances, the more recent findings and interpretations of different observers have been remarkably consistent.

For example, Smiley, in a statistical analysis of the effect of seasonal factors on the incidence of acute respiratory infection, concludes that these infections vary inversely with mean outside temperature and hours of sunlight (4). Chapin, analyzing 102 cases of pneumococcus infection at the Pennsylvania Hospital, notes a relatively high incidence of Type I infection during the winter and early spring months (5). "Common colds" among a group of 6700 clerical employees of

the Metropolitan Life Insurance Company were found to have "two periods of maximum incidence; the first, following the advent of cool weather in the late summer and early fall; the second, occurring during the following January or February when the coldest weather of winter prevails" (6). Schütz in an extensive study of the epidemiology of measles reports that in the large cities of Schleswig-Holstein, the greatest number of cases occur during the spring and fall (7). Pope finds the greatest incidence of scarlet fever to be in January and lowest in July and August but does not think there is evidence of "trend or definite seasonal variation" (8). Rogers attributes the seasonal fluctuations of smallpox in India to the degree of absolute humidity (9). Brown, Pearce, and Van Allen analyzed the first twenty generations of a transplanted rabbit tumor and found that variations in its malignancy tended to follow a seasonal periodicity (10). This they interpreted as due to seasonal fluctuations in animal resistance. Furthermore, by compiling the autopsy data on a large number of so called "normal" rabbits, they found that body and organ weights undergo a definite cyclic change associated with the season (11).

Racial differences in infant mortality in the United States have been studied recently by DePorte. He concludes that the differences in rates of mortality of infants under 1 month of age "very probably have a biological basis and, in this sense, may be termed racial" (12). Meyer and Burghard analyzed the occurrence of scarlet fever and found that family and individual anlages are exhibited definitely by similarity of infection and that the constitution of the individual determines his susceptibility to infection and the course and severity of his disease (13). Lewis (14) and Stevens (15) have noted the same apparent differences in susceptibility to scarlet fever. By means of a skin test with a Shiga dysentery toxin, Brokman and Przesmycki examined 800 individuals for sensitivity. They state that the mechanism of physiological immunity in dysentery is the same as that of diphtheria. It consists in the hereditary capacity to provide normal antitoxin. It is probably a general biological principle which regulates the mechanism of physiological individual immunity against toxogenic bacteria (16). Finally, in a genetic and biometric study of the "Constitutional element in the etiology of pneumonia," Pearl (17) describes a pedigree which has in the father's kinship—"a group of people with a definite tendency towards constitutional inferiority of the respiratory system, which manifests itself chiefly in a tendency to break down from pulmonary tuberculosis in early adult life. This particular constitutional inferiority is absent in the mother's kinship, but in that group of people there is definitely manifest a constitutional tendency to generally non-fatal respiratory infections, bronchitis and bronchopneumonia, in infancy and childhood. When these two constitutional traits were combined, by the mating of the father and mother of the 13 children in the sibship under discussion, there was produced a group of children with extremely low resistance to any sort of respiratory infection, with a consequent 100 per cent incidence of pneumonia in the years of infancy and childhood."

These references to recent literature will perhaps indicate the present trend of thought on these problems. The data are with one exception, which follows, purely descriptive, and have inherent in them a number of indeterminable variables which for the most part have been dealt with according to statistical methods. A direct experimental proof of seasonal variation in mouse typhoid infection has, however, been offered, under conditions in which every known variable except season has been eliminated or controlled (3, *a*). Also racial differences in susceptibility to this infection have been proved experimentally (3, *b*) as well as wide differences in the susceptibility of individuals (1, *h*). Tests with *Bact. lepisepticum* have also furnished evidence that individual rabbits differ in their susceptibility to this disease (1, *i*). Hence the descriptive data are in no small measure supported by the more precise results of direct experiment.

From the general epidemiological view-point, the observations recorded in this paper are of interest in that they describe a rabbit community in which a bacterial infection was endemic and where the type and virulence of the microorganisms concerned were tested from time to time and found to be uniform and constant. A similar study of a rabbit community in which a severe epidemic occurred is reported in the following paper by D. T. Smith (2). Types of *Bact. lepisepticum* recovered by him at Saranac resembled in every respect those occurring at New City. Okamoto, studying epidemics among laboratory animals, found bacterial virulence to be relatively constant (18). R. Freund, investigating a mixed Pasteurella, pneumococcus, and paratyphoid epidemic among a stock of rabbits and guinea pigs at the Robert Koch Institute in Berlin, found no differences in the virulence of various epidemic strains, and concluded that the cause of the outbreaks was due to an increase in population susceptibility following sudden and severe changes in temperature (19). Finally, Theobald Smith and his collaborators have studied a paratyphoid epidemic among a stock of guinea pigs and found no definite fluctuations or differences in the virulence of the specific bacteria. They, too, conclude that changes in population susceptibility must be regarded as the most probable cause of the outbreak (20). Several populations, therefore, have been observed by different investigators to be infected with intestinal and respiratory diseases which have

undergone epidemic and endemic fluctuations in prevalence and severity without any demonstrable change in the virulence or type of the bacteria concerned.

SUMMARY.

The spread of *Bact. lepisepticum* infection throughout a population of thoroughbred rabbits at a farm in New City, N. Y., was studied over a period of 2 years. The following observations are noteworthy.

1. 50 to 75 per cent of the total mortality was due to *Bact. lepisepticum* infection.

2. About 50 per cent of the stock was affected locally with *Bact. lepisepticum* "snuffles."

3. "Snuffles" and mortality rates showed spring and fall rises and low points during the summer.

4. Carrier, morbidity, and mortality rates of various special breeds differed consistently and to a marked degree. All reflected the same seasonal fluctuations as the total population.

5. Individuals within each breed differed in their response to the presence of *Bact. lepisepticum;* some died of penumonia and septicemia; others developed the local nasal infection, "snuffles;" others became "healthy" carriers; and a few remained uninfected.

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EPIDEMIOLOGICAL STUDIES ON RESPIRATORY INFECTIONS OF THE RABBIT.

X. A Spontaneous Epidemic of Pneumonia and Snuffles Caused by Bacterium lepisepticum among a Stock of Rabbits at Saranac Lake, N. Y.

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During the winter of 1925, we had an opportunity to observe and study a spontaneous epidemic of *Bact. lepisepticum* pneumonia and snuffles among the stock rabbits at the Saranac Lake Laboratory, N. Y. The circumstances were favorable, since we had some preliminary knowledge, both of the bacteria and of the rabbit populations concerned, and were thus able to determine that throughout the period microbic virulence remained constant and that host susceptibility differences were the predisposing cause of the outbreak. The course of events and descriptive data are given in the following paragraphs.

The Saranac Lake Laboratory for the Study of Tuberculosis maintains a stock of rabbits of mixed Belgian hare breed which had been entirely free from respiratory infection for a period of over 2 years. These animals were either purchased or reared of stock obtained from farmers in the Adirondack Mountains. During the 6 months preceding this epidemic, over 100 rabbits were used by Dr. Willard Soper in a study of experimental tuberculous meningitis (1) and none of the animals developed respiratory infection although the induced disease often ran a chronic course and the infected ones became very weak and much emaciated before death.

At the New York State Hospital for Incipient Tuberculosis at Ray Brook, there existed a stock of rabbits heavily infected with both *Bact. lepisepticum* and *B. bronchisepticus*. In October, 1924, about

fifteen of these rabbits were transferred to the Saranac Lake Laboratory. On arrival, they proved to have snuffles and were therefore isolated from the normal stock. The same attendant fed both the normal Saranac stock and the infected rabbits from Ray Brook, but he did not handle the infected ones or carry them in the baskets used for

TABLE I.

Results of Autopsies on Infected Ray Brook Rabbits before Onset of Epidemic.

No. of rabbit	Presence of snuffles	Bacteriology of nasal passages	Bacteriology of middle ears	
			Right	Left
1	+	*Bact. lep.*	*Bact. lep.*	*Bact. lep.*
2	+	" "	" "	" "
3	+	" "	" "	" "
		B. bronch.		

Bact. lep. = *Bacterium lepisepticum.*
B. bronch. = *Bacillus bronchisepticus.*

TABLE II.

Results of Autopsies on Infected Ray Brook Rabbits after the Onset of the Epidemic.

No. of rabbit	Presence of snuffles	Bacteriology of nasal passages	Bacteriology of middle ears	
			Right	Left
1	+	*Bact. lep.*	*Bact. lep.*	*Bact. lep.*
		B. bronch.		*B. bronch.*
2	+	*Bact. lep.*	" "	
		B. bronch.	*B. bronch.*	0
3	+	*Bact. lep.*	*Bact. lep.*	*Bact. lep.*
		B. bronch.	*B. bronch.*	*B. bronch.*

Bact. lep. = *Bacterium lepisepticum.*
B. bronch. = *Bacillus bronchisepticus.*

the normal stock. Under these conditions the disease did not spread. A few of the carriers died and were cremated without autopsy, but most of the infected animals remained in excellent condition, as shown by their heavy weight and their smooth, glossy fur.

On February 2, 1925, 5 months later, it was decided to kill the infected animals because they were considered useless for experimental

work. Three of the carriers were autopsied. The sinuses and both middle ear cavities were found filled with pus. Cultures were made on blood agar plates after the method described by Webster (2), and a pure culture of *Bact. lepisepticum* was obtained from the nasal passages and each middle ear of two of the rabbits. From the third animal a pure culture of *Bact. lepisepticum* was found in the middle ears, but in the nasal passages a mixture of *Bact. lepisepticum* and *B. bronchisepticus* was present. Subsequent autopsies and bacteriological cultures on three others showed both organisms in the nose (Tables I and II). It is significant that all six carriers had the form of *Bact. lepisepticum* which Webster (3) has identified as the mucoid type.

Through an error, the carriers were placed in the baskets that were reserved for the normal Saranac stock animals and they remained there for some hours before being transferred back to their cages. The baskets were then used again for the normal animals without being sterilized. Within a week the caretaker noticed that some of the twenty-two normal Saranac stock rabbits were beginning to sneeze. The symptoms of "snuffles," which were mild at first, became more severe and on February 20, 1925, which was 18 days after the accidental contamination of the baskets, the first of the normal rabbits died. The autopsy showed pneumonic consolidation of both lungs and a small amount of seropurulent fluid in the pleural cavities. Cultures and smears of both lungs and pleural fluid revealed *Bact. lepisepticum* in large numbers and in pure culture. During the next 15 days, six other rabbits died of pneumonia. These were all examined, except two, which unfortunately were cremated without autopsy. Those studied showed consolidation of one or both lungs and constant involvement of the pleura. Pure cultures of *Bact. lepisepticum* were found in each case. Some animals had complicating infections of the sinuses and middle ears, while others did not (Table III).

Following this period of high death rate, there was a lull in the epidemic. One animal, which had heavy, labored respiration, continued in that state for 3 weeks and then died. The autopsy revealed a pneumonic process complicated by an empyema on one side only, the other lung being essentially normal. *Bact. lepisepticum* was isolated in pure culture from the right lung, the right pleural cavity, and the right middle ear, while *Bact. lepisepticum* and *B. bronchisepticus* were

TABLE III.

Results of Autopsies on Saranac Rabbits during and after Epidemic.

No. of rabbit.	Fate of rabbits	Presence of snuffles	Bacteriology of nasal passages	Bacteriology of middle ears		Bacteriology of lungs	Remarks
				Right	Left		
1	Died	−	−	−	−	*Bact. lep.*	Pneumonia
2	"	−	−	−	−	" "	"
3	"	+	*Bact. lep.*	0	*Bact. lep*	" "	Pleuropneumonia
4	"	0	0	*Bact. lep.*	0	" "	"
5	"	+	*Bact. lep.*	0	*Bact. lep.*	" "	"
6	"	+	" " and *B. bronch.*	*Bact. lep.*	0	" "	Pneumonia and empyema
7	Killed	+	*Bact. lep.* and *Staph. A.*	0	*Bact. lep.*	0	
8	"	+	*Bact. lep.*	*Bact. lep.* *B. bronch.*	" " *B. bronch.*	0	
9	"	+	" " and *B. bronch.*	0	*Bact. lep.*	0	
10	"	+	*Bact. lep.* and *Staph. A.*	*Bact. lep.*	0	0	
11	"	+	*Bact. lep.*	" "	*Bact. lep.*	0	
12	"	+	" "	" "	" "	0	
13	"	+	" "	" "	0	0	
14	"	+	" "	0	0	0	
15	"	+	" " and *B. bronch.*	0	0	0	
16	"	+	*Bact. lep.* and *B. bronch.*	0	0	0	

17	Killed	+	*Bact. lep.*	0	0	0	*Bact. lep.* from subcutaneous abscess
18	"	+	" "	0	0	0	" "
19	"	+	" "	0	0	0	Eye infected with *B. bronch.*
20	"	0	0	0	0	0	Normal.

Bact. lep. = *Bacterium lepisepticum.*
B. bronch. = *Bacillus bronchisepticus.*
Staph. A. = *Staphylococcus albus.*

found in the nasal passages. This was the last of the animals to die of the infection.

At this time the remaining fourteen rabbits were in poor physical condition. They showed marked loss of weight, the hair was rough and untidy, and one had an infection of the eye; another had a large abscess on the buttock, still another had multiple subcutaneous abscesses, and thirteen of the fourteen animals showed clinical evidence of snuffles. The rabbit with the eye infection and the two with abscesses were killed and autopsied. The remaining eleven, under the influence of good diet and care, began to improve rapidly, gained weight, became more active, and within 6 weeks seemed to be normal except for the presence of pus in the nostrils and the matting of the hair on the fore feet. When it became evident that no more rabbits would succumb, the remainder of the group was killed and autopsied, and as shown in Table III, only one of the original twenty-two animals was entirely free from infection at the time of the autopsy, and even this one had clinical snuffles at the height of the epidemic.

Cultures of *Bact. lepisepticum* obtained before, during, and after the epidemic appeared to agree in morphology, colony formation, and cultural reactions. Strains taken from Ray Brook rabbits before the onset of the epidemic and from Saranac rabbits dying of pneumonia during its peak were sent to Dr. Webster at The Rockefeller Institute and found by him to be of the mucoid type, identical with other mucoid forms in cultural reactions and virulence.

SUMMARY.

These observations have brought out three facts which may be stated briefly as follows.

1. An acute respiratory epidemic was incited by mucoid strains of *Bact. lepisepticum* of moderate and uniform virulence.

2. The epidemic was brought about not by an alteration in the virulence of the prevailing strains of *Bact. lepisepticum* but by a sudden distribution of virulent organisms among a susceptible population hitherto unexposed.

3. Individuals in this population showed different degrees of resistance to the infecting bacillus; some died from pneumonia and septicemia, while others localized the infection to the nasal passages.

The significance of these facts has been discussed at length elsewhere (4).

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ON THE COLLECTION OF THE ENTIRE EXTERNAL SECRETION OF THE PANCREAS UNDER STERILE CONDITIONS AND THE FATAL EFFECT OF TOTAL LOSS OF PANCREATIC JUICE.

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Though de Graaf first put a cannula into the pancreatic duct and collected pancreatic juice in 1664 (1), our knowledge of this secretion really dates from the epoch-making discoveries of Claude Bernard (1849) (2), who studied in great detail the juice obtained by placing a silver cannula joined to a rubber tube into the large pancreatic duct. He found, and others after him, that such a method yields pancreatic secretion for only a few days, since infection soon leads to the sloughing of the cannula and the rapid closure of the fistula thus left, the secretion either draining through accessory ducts or newly made channels back to the duodenum.

The successful establishment of a more or less permanent pancreatic fistula was performed by Pavlow (3). Bernstein (4) before him had tried to keep an open channel for the flow of pancreatic juice by inserting lead wires into the duct, but this did not succeed. Pavlow transplanted the entire pancreatic duct together with a rhomboidal piece of duodenal mucous membrane surrounding its orifice into the anterior abdominal wall, closing the buttonhole opening in the duodenum with sutures. Heidenhain described a similar method; but he excised the entire circumference of the bowel, including the opening of the duct, anastomosed the cut intestines, and transplanted the duct as Pavlow did.

In either event, the subsequent course of the operation proved troublesome, for the secretion quickly excoriated the edges of the wound and, even with special precautions, frequently led to the digestion of the anterior abdominal wall (5). Though pancreatic juice, as secreted, has little or no proteolytic power, it was soon demonstrated by one of Pavlow's pupils, Schepowalnikow (6), that the inactive trypsinogen was activated by enterokinase, a secretion of the duodenal mucosa surrounding the duct opening. The difficulty was finally obviated by Babkin (7) who excised the offending duodenal tissue. In this way, a tight scar was formed at the opening of the fistula so that, at times when secretion was desired, a metal or glass catheter was introduced and the juice collected, while at other times it was forced to follow other channels back to the duodenum.

Others have modified the technique of Pavlow and his pupils by isolating the pancreatic duct outside the duodenum, ligating, cutting and sewing the distal end into the anterior abdominal wall. It appears to have been first described by Frouin (8) in 1913 and again by Inlow (9) in 1921, but it was really Senn (10) in 1886 who originally performed this operation and collected pancreatic juice in this way. He was able to collect as much as 120 cc. per day but found that the amount secreted quickly diminished and finally ceased.

Still another type of pancreatic fistula has been described by Fodera (11) who placed a T-tube into the pancreatic duct so that the juice could either go on directly to the duodenum or through one arm of the T to the outside. It is similar in principle to the "amphibilous" biliary fistula described by Schiff (12). But it has never been used by others, apparently, and little is known of its merits.

The general objection, however, to all open fistulas for the collection of pancreatic juice is the impossibility of obtaining sterile secretions. Infection leads to marked alterations of the properties of pancreatic juice and moreover travels up into the ducts and brings about inflammatory changes in the gland itself, and frequently in other organs (13). With an open fistula it is difficult or impossible to prevent licking of the secretions by the animal so that one can only guess how much of the juice is returned to the body and how much is lost.

Similar objections apply to the collection of bile through an open biliary fistula. Rous and McMaster (14) in 1923 described an ingenious operation whereby the total bile was collected under sterile conditions for many months. In our studies of the pancreatic juice we have employed this type of intubation, as described in detail below.

Experimental Procedure.

Under ether anesthesia and with the most scrupulous aseptic precautions, the duodenum was brought into a right rectus incision and the accessory pancreatic duct located on its dorsal side close to where the common duct ends. This duct was ligated and cut and the dissection continued downward till the large duct was reached.

As pointed out by Hess (15), there may be more than two pancreatic ducts in the dog. If there are, they empty into the duodenum between the two largest ones mentioned above. In dissecting the head of the pancreas away from the duodenum, therefore, we were assured of having cut all of them. During the course of our operations, however, we encountered such additional ducts only a few times, and they were very small.

The large duct was dissected from the surrounding tissue and two small arteries which usually accompany it were tied and cut in order to prevent troublesome bleeding later. The duct was then tied close to the duodenum, from the ventral side, a small incision made and the cannula inserted and tied in place. The duct was cut across between the point of intubation and the duodenum. Omentum was tucked between the head of the pancreas and the gut to insure against reconstitution, as well as to cover the raw areas exposed by the dissection.

The collecting tubes were prepared beforehand and autoclaved twice in small Erlenmeyer flasks. They consisted of two parts, one of soft compressible "hemocytometer" tubing (*E* and *A*, Fig. 1), the other of harder and firmer material (*C* and *F*, Fig. 1). The former was attached to the cannula and, being soft, conformed more or less readily to the intraabdominal movements without kinking, the latter emerged from the abdomen through a separate opening in the side and, being harder, was not so easily compressed. The two were joined by a curved glass connection. The tubes were placed down into the pelvis before

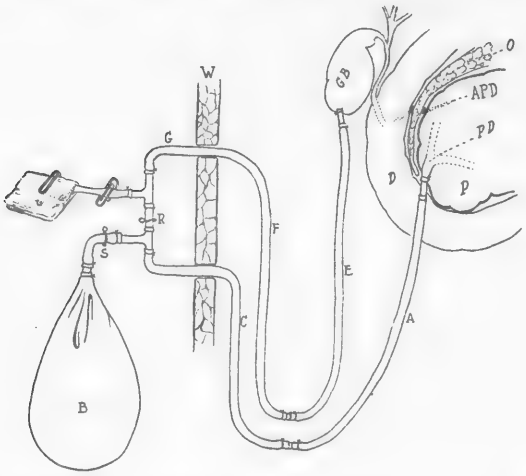

FIG. 1. "Altercursive" intubation of the pancreatic duct. The accessory pancreatic duct (*APD*) is cut and omentum (*O*) is tucked between the head of the pancreas (*P*) and the duodenum (*D*). Pancreatic juice flows from the main pancreatic duct (*PD*) through the efferent tube (*A–C*) into the rubber collecting bag (*B*) whenever the clamp (*R*) is closed. When the clamp (*S*) is closed on the other hand juice flows back through the afferent tube (*G–F–E*) into the gall bladder (*GB*) and through the common duct to the duodenum. (See text.)

the abdomen was closed, so that they formed a large U before emerging to the outside. The omentum so adheres to this length of tubing that infection does not extend upward and the collecting system may remain uninfected very often for weeks or months in the absence of other complications.

The emerging tube was attached to a T-tube of glass, the other two arms of which were joined to the collecting balloon and the outlet tube all of which had been previously sterilized by autoclaving. All joints were covered with gauze soaked in 5 per cent phenol.

It was found that the preoperative feeding of the animal was important. All food was withheld for 24 to 48 hours, but, about 5 hours before operation, 200 gm. of finely ground meat was given which acted as a stimulant to pancreatic flow so that when the duct was incised juice poured out profusely thus facilitating intubation and insuring the continuance of secretion afterward without accidental obstruction due to kinking.

Aseptic precautions were maintained particularly during the collection of the juice which was prone to infection by contact or contamination from the air. Collections were made as quickly as possible into sterile containers and the outlet tube carefully covered with several layers of sterile gauze soaked in 5 per cent phenol. The secretion was frequently cultured and the sediment from centrifugalized specimens stained for bacteria. When infection occurred, the animal was either killed or used for other special studies.

EXPERIMENTAL FINDINGS.

We shall here be concerned with those instances in which the flow of pancreatic juice continued uninterruptedly after operation.

In a good many experiments this did not occur. Frequently, the secretion became infected from contact or contamination from the air during periods of collection. In this event, the flow diminished or even ceased entirely in most cases after the lapse of several days. In other instances obstruction developed due, most commonly, to a kink in the rubber collecting tube, a twist in the bag or to the duct's turning on itself so as to close the opening of the cannula. The findings, when such complications took place, proved of much interest and will be described in subsequent reports.

When obstruction or infection did not occur, the secretion continued to flow profusely and, after 5 to 8 days, quite to our surprise, the animals died with marked asthenia, as described briefly in a previous paper (16). In all, twelve dogs were studied in which this fatal outcome was observed.

It is of interest to note that, according to Babkin (5), Bernard, Pavlow, Heidenhain and others observed hypersecretion followed by death in dogs with an open pancreatic fistula. It was regarded as an exceptional occurrence. In our experiments, where the secretion remained sterile and unobstructed, it was the rule.

By the next day after the operation, which usually consumed about an hour, pancreatic juice was found in the collecting balloon and by the 2nd day, the amount had increased. The dog was healthy, active and eating well. Though the secretion was at first slightly turbid, by the

2nd day it had assumed its normal appearance—colorless, very slightly opalescent and quite odorless. It was alkaline enough to turn phenolphthalein a faint pink and when titrated with acid, bubbles of carbon dioxide were given off. With Töpfer's reagent as an indicator, it would neutralize more than its own volume of N/10 HCl, the usual value being .11 to .12 N. It contained a large amount of coagulable protein. Amylase and lipase were present in active form. Casein was very slowly digested unless a drop or two of duodenal extract were added when it was dissolved almost instantly.

For 2 days or 3, the animals thus deprived of the total external secretion of the pancreas appeared normal in every way. But then the appetite rapidly diminished. They ate their daily ration of cooked meat, biscuit and milk but instead of eating heartily in gulps as usual, they took longer and often consumed the meal only after several hours or all day. By the 4th day, food was refused entirely and even milk given by gavage was returned. Large amounts of water were taken but soon it was not tolerated. By the 5th or 6th day vomiting had become marked whether fluid was swallowed or not, in the latter case only mucus and froth appearing. The stools, at first bulky and soft, soon diminished in volume and became scanty and loose, although always well pigmented.

Asthenia was noted early and became a prominent symptom. The animal, though in no distress, became increasingly weak and unsteady, reminding one of the asthenia observed after adrenalectomy. But the temperature was not subnormal and respirations and pulse rate were unchanged. By the 6th to 8th day, the animal died. Convulsions were not observed.

The secretion of pancreatic juice continued up to the very end in amounts dependent somewhat upon the size of the dog but apparently in no relation to the intake of food since the largest amounts were secreted in the later days of the experiment when no food was taken at all (see Table I), and while vomiting was persistent. In Dogs VIII and IX, for example, 475 cc. of secretion were secreted during the 24 hours preceding death.

At autopsy, performed in most instances immediately after death, no gross changes were observed. The peritoneal cavity was everywhere smooth and glistening and the operative wound was healed

per primam. The long outlet tube was firmly fixed by omentum and no infection anywhere seen. The pancreas seemed somewhat smaller than normal but was soft and pink. The glass cannula was tightly bound to the duct by fibrous adhesions. The liver and biliary system appeared normal. The stomach was small and firmly contracted particularly the pyloric portion. Its mucous membrane was thrown into deep folds, was hyperemic and showed small erosions or ulcerations. Microscopic sections of the pancreas showed small contracted

TABLE I.

The Amounts of Pancreatic Juice (in C.c) Secreted Each 24 Hours Following Drainage of the Total Secretion, until Death.

Dog No.	Weight	Days								
		1	2	3	4	5	6	7	8	9
	kg.									
I	15	175	275	300	325	325				
II	9	30	170	290	275	250				
III	9	17	185	300	250	185	155			
IV	14	95	260	340	330	440	405	105		
V	16	275	340	245	340	230	245	258	135	
VI	18	25	420	625	590	525	320			
VII	14	275	285	325	460	325	200			
VIII	10	205	280	265	140	315	330	360	475	
IX	11	310	425	205	215	170	210	320	475	
X	10	225	110	200	30	290	300			
XI	14	130	310	300	Obstructed	375	250	125		
XII	12	100	175	210	180	280	305			

acini surrounded by rather wide clear spaces, in distinct contrast to their plump full appearance in the normal gland. The islets of Langerhans seemed unchanged. The adrenals revealed no abnormality.

Blood changes were observed in many cases and will be reported in detail subsequently. In all a marked dehydration was demonstrable by taking hematocrit readings with the Van Allen tube (17). The proportion of red cells to whole blood often reached 80 per cent, the normal being 40 to 50 per cent. There was a decrease in blood chlorides and an increase in pH. Blood sugar determinations were within

normal limits.* The urine was repeatedly examined for reducing bodies but none found.

"Altercursive" Intubation of the Pancreatic Duct.

For the permanent study of the total pancreatic juice, it was necessary to provide for the return of the secretion to the duodenum at will so that the fatal effect of its continued loss might be avoided and still its drainage at times of observation be possible. Such an experimental animal can be prepared by using two tubes instead of one, the second tube being used to return the outflowing secretion through a cannula intubated into the duodenal end of the severed duct.

In the case of bile such a method has already been described by McMaster and Elman (18), and successfully used by them in many experimental studies. The same technique was employed for the pancreatic juice. In this way several dogs were prepared and kept alive for several weeks, and in one case for 2 months, by alternately shifting the secretion to the bag and to the duodenum. If, at any time, the juice were allowed to flow off continuously, the same fatal course followed as described above. On the other hand, two dogs, almost moribund from continued loss of pancreatic juice, were quickly restored to normal by merely turning the flow back to the duodenum.

There are difficulties, however, with the type of altercursive intubation mentioned above, as applied to the pancreatic duct. Frequently, the main duct is so short that there is not enough tissue for the insertion of two cannulas. Attempts to use the accessory duct to return the secretion failed because of the small size of this channel. The most serious obstacle, however, was the difficulty in maintaining sterility of the secretion for very long periods of time. Perhaps it was due to the shortness of the duct which allowed intestinal bacteria to penetrate. In the case of the common duct there appears to be a sufficient protection due probably to its length and the effectiveness of the sphincter of Oddi. Thus, McMaster and Elman (18) found that, with an altercursive intubation of the common duct, the bile remained sterile for months.

* These determinations were made by Dr. Ronzoni.

It is likely that the difficulty in the case of pancreatic juice lies in the fact that fecal contamination is more readily possible. We have noted that fecal contents are always present in the stomach of dogs draining the total pancreatic juice and it is therefore easy to see how such infective material could easily ascend the short length of duct and reach the collecting tubes.

Another attempt was made by returning the pancreatic juice through the biliary system (see Fig. 1). That is, the tube by which the secretion is to be carried back to the duodenum was inserted into the gall bladder or into the cystic duct after removal of the gall bladder. Two successful instances were possible, no ill effects apparently following the admixture of bile and sterile pancreatic juice. More experiments must be performed, however.

DISCUSSION.

It has been possible by appropriate intubation of the pancreatic duct, by a method described above, to obtain for study the entire external secretion of the pancreas under sterile conditions. The secretion thus obtained is abundant in amount, slightly opalescent in appearance and without odor. It is decidedly alkaline in reaction and contains a large amount of protein, and active lipolytic and amylolytic ferments. Trypsin is present but in the inactive form so that it exerts little proteolytic action. On the addition of a drop or two of duodenal extract it digests protein rapidly.

The fact that such drainage of the total pancreatic juice uniformly leads, in absence of infection or obstruction, to the death of the animal in 5 to 8 days brings up a number of important questions in the mechanism of the external secretion of the pancreas and concerning its relation to the stomach.

The stimulus to this seemingly intense secretion must be one quite apart from the ingestion of food since it continues long after food is refused. The acid gastric juice immediately suggests itself since it is normally a most powerful stimulant of pancreatic secretion. Indeed, we may imagine the gastric acidity as constantly effective under the conditions of the experiment, even in the absence of food, if we assume that the regurgitation of alkaline pancreatic juice into the stomach normally occurs as a regulator of gastric acidity. The drain-

age of the pancreatic juice to the outside thus gives rise to the unopposed acid secretion and a vicious circle is formed quickly leading to the death of the animal since, by the vomiting, the loss of gastric juice is added to that of the pancreatic juice.

From the blood findings mentioned above and from the fact that death occurs on the 6th to 8th day—about the duration of life after acute pyloric obstruction—a possible analogy between the two conditions may exist. The persistent vomiting in itself may be responsible for these findings since Gamble (19) has shown that loss of fixed base in the vomitus brings about the alkalosis and lowered blood chlorides. That the death may be analogous to that following the establishment of a duodenal fistula seems possible. Hartmann (20) has shown that the loss of fixed base in this condition leads to the same blood findings as that following persistent vomiting. The absence of glycosuria and abnormal blood sugar values would seem to rule out any involvement of the internal secretion of the pancreas.

The reason for the persistent vomiting may be connected with the elimination of the regurgitation of the alkaline pancreatic juice into the stomach, which is claimed to be a normal and from our findings might be an indispensable phenomenon. We have frequently noted in our animals that the giving of 0.5 per cent acid solutions into the stomach after the 3rd or 4th day of drainage would promptly result in vomiting even though tap water would be tolerated. Boldyreff (21), in similar experiments noted "the toxic effect of acid solutions in the stomach" of dogs draining pancreatic juice. The prompt restoration of a moribund animal to a practically normal condition by the simple procedure of turning pancreatic juice back into the duodenum suggests the possibility of the presence in the juice of a substance necessary for life.

SUMMARY.

It has been possible by appropriate intubation of the main pancreatic duct of the dog to collect the total external secretion of the pancreas under sterile conditions. When all of the secretion is thus collected exitus occurs in a characteristic way in about a week with anorexia, gastric irritability, vomiting and asthenia. The significance of this finding has been briefly discussed.

A method is also described for the "altercursive" intubation of the pancreas whereby the secretion may be collected or allowed to flow back to the duodenum at will.

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